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Experimental study of micro-habitat selection by ixodid ticks feeding on avian hosts

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ABSTRACT

Mechanisms of on-host habitat selection of parasites are important to the understanding of host-parasite interactions and evolution. To this end, it is important to separate the factors driving parasite micro-habitat selection from those resulting from host anti-parasite behaviour. We experimentally investigated whether tick infestation patterns on songbirds are the result of an active choice by the ticks themselves, or the outcome of songbird grooming behaviour. Attachment patterns of three ixodid tick species with different ecologies and host specificities were studied on avian hosts. *Ixodes arboricola*, *Ixodes ricinus* and *Ixodes frontalis* were put on the head, belly and back of adult great tits (*Parus major*) and adult domestic canaries (*Serinus canaria domestica*) which were either restricted or not in their grooming capabilities. Without exception, ticks were eventually found on a bird's head. When we gave ticks full opportunities to attach on other body parts – in the absence of host grooming – they showed lower attachment success. Moreover, ticks moved from these other body parts to the host's head when given the opportunity. This study provides evidence that the commonly observed pattern of ticks feeding on songbirds' heads is the result of an adaptive behavioural strategy. Experimental data on a novel host species, the domestic canary, and a consistent number of published field observations, strongly support this hypothesis. We address some proximate and ultimate causes that may explain parasite preference for this body part in songbirds. The link found between parasite micro-habitat preference and host anti-parasite behaviour provides further insight into the mechanisms driving ectoparasite aggregation, which is important for the population dynamics of hosts, ectoparasites and the micro-pathogens for which they are vectors.

1. Introduction

Parasitic species show a wide variation in host specificity (Poulin, 2011) and even when parasitizing different host species, parasites can be quite specific to well-defined microhabitats, i.e. infesting specific body parts in or on the host (Adamson and Caira, 1994). Different optimizing principles can drive adaptations to a narrowed ecological niche (Templeton and Rothman, 1974) with multiple selective forces, trade-offs and constraints (e.g. genetic background, phenotypic plasticity) accounting for the formation and retention of site specificity (Ebert, 1998; Little et al., 2006; Leggett et al., 2013). Unfortunately, we still have limited knowledge about the relative importance of factors shaping micro-habitat selection and parasite distribution in or on hosts. Factors such as a parasite's nutritional needs, parasite size and mobility, and its ability to circumvent the behavioural and physiological defences of the host are among the most likely determinants which play a role in micro-habitat choice (Downes, 1989; Poulin, 2011). Micro-habitat preferences might also drive or maintain selection for a specific body part. For example, a recent investigation in passerines belonging to several different families found that feathers on the head are shorter and create a more shallow layer compared with feathers on the back and belly (Strubbe, 2019). This might generate micro-habitat differences between body parts that are selected for by ectoparasites.

Ectoparasites are a particularly interesting group in this respect because they can potentially feed on many different locations on the host, yet often their distribution on the body surface is rather narrow. Ticks are no exception as they are generally only found on specific parts of the host's body (Balashov, 1972) with attachment sites differing greatly between host, parasite species and life stage.

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Among blood-sucking ectoparasites, ticks are of great veterinary and medical importance as they transmit a vast array of pathogens such as bacteria, protozoans, viruses and fungi (Sonenshine et al., 2002; Goodman et al., 2005; Sonenshine and Roe, 2013). Extant ticks are divided into two major clades, Argasidae (soft ticks) and Ixodidae (hard ticks), with Nuttalliellidae as an additional monotypic group. Argasid (Argasidae) and ixodid (Ixodidae) ticks have different feeding characteristics with the Ixodidae spending more time attached to the host skin and feeding only once for each instar (Binnington and Kemp, 1980; Uspensky, 2008). In this study we focus on the distribution of ixodid ticks on birds.

Observational and experimental studies in mammals and reptiles typically reported a non-random distribution of tick attachment sites. The body parts to which hard ticks attach vary between tick species (Andrews et al., 1982; Felz and Durden, 1999). Different instars of the same species can also attach to different body parts (Koch, 1982; Dantas-Torres and Otranto, 2011; Kiffner et al., 2011) and no published overall attachment pattern for different tick species parasitizing mammals or reptiles has been described to date.

In birds, most observational data show a consistent pattern of attachment: ixodid ticks are typically found engorging on a host's head, in particular on the face, ear, eyelid, and crown (Walter et al., 1979). This is true regardless of the ecology and distribution of the bird species (Table 1). Data in the literature show a strikingly similar attachment pattern in different tick species such as *Ixodes ricinus*, *Ixodes lividus* or *Ixodes auritulus,* despite infesting birds as different in distribution, ecology and taxonomy as the pheasant (*Phasianus colchicus*; Hoodless et al., 2003) or the rufous-capped spinetail (*Synallaxis ruficapilla*; Arzua et al., 2003). Nevertheless, not all bird and tick species show this typical pattern of attachment. In fact *Ixodes uriae*, the most widespread tick parasitizing a wide range of seabirds, shows very different sites of attachment (Table 1). For instance, in King penguins (*Aptenodytes patagonicus*), *I. uriae* adults and nymphs feed on the head and neck while larvae mostly attach to the lower parts of the body (Gauthier-Clerc et al., 1998). In common murre (*Uria aalge*), thick-billed murre (*Uria lomvia*), black-legged kittiwake (*Rissa tridactyla*), and red-legged kittiwake (*Rissa brevirostris*), *I. uriae* was found on many different body parts such as back, belly and tail (Choe and Kim, 1988; Danchin, 1992; Barton et al., 1995) while all *I. uriae* ticks were attached to the plantar surface of the foot web in Cassin's auklets (*Ptychoramphus aleuticus*; Morbey, 1996).

An obvious hypothesis to explain the observed pattern of tick attachment in birds is that ticks aggregate on the head because there they are least vulnerable to grooming. Grooming is one of the most important defences against ectoparasites (Clayton et al., 2010; Bush and Clayton, 2018) with evidence of selective pressures acting on both hosts and parasites (Clayton et al., 2015). Nevertheless, we are not aware of any experimental studies that have explicitly tested this hypothesis. More importantly, it is unknown whether this aggregation on the head is driven by the ticks' behavioural preference (which has been shown to drive differences in attachment to individual nestlings; Heylen and Matthysen, 2011), or by the selective removal of ticks from other body parts, performed by the host. In addition, if the body part for attachment is mainly determined by tick preference, then we might expect a less specific and confined attachment area for more generalist tick species due to their adaptation to feed under a wider range of different conditions. In this paper we experimentally test whether the non-random tick infestation patterns observed in wild songbirds is the result of the tick's preference for the host's head or results from host grooming. To that end we administered three species of ixodid ticks, which differ in ecology and host specificity (Heylen et al., 2014a), to different host body parts using two bird species. We compared tick attachment patterns between hosts that were or were not able to groom. If ticks' behaviour drives attachment patterns then ticks should be found engorging on the host's head regardless of where they were placed; if birds' behaviour influences tick attachment patterns, tick distribution should differ between birds that were restrained from grooming and those that were not. Clearly, both host and parasite behaviour could influence tick attachment patterns.

2. Materials and methods

2.1. Study system

For this study we used the three ixodid tick species that are the most common on European songbirds. These three species strongly differ in habitat requirements, host specificity, and phenology (Heylen et al., 2014a). The tree-hole tick, *Ixodes arboricola* Schulze and Schlottke 1929, is an endophilic bird-specialised hard tick. Its entire life cycle takes place in cavities and it relies on its host to spread to other tree holes and nests (Van Oosten et al., 2014). The main hosts of *I. arboricola* are shared with *I. ricinus* (Linnaeus 1758), an exophilic ground-dwelling tick. The latter can be found on an extensive range of vertebrates although adults mainly parasitise large mammals (Humair et al., 1993; Olsén et al., 1995; Comstedt et al., 2006). *Ixodes ricinus* transmits many pathogens such as bacteria (e.g. *Borrelia burgdorferi* sensu lato, *Rickettsia* spp.), viruses (e.g. tick-borne encephalitis virus) and protozoans (e.g. *Babesia* spp., *Trypanosoma* spp.) (Sonenshine and Roe, 2013). Finally, *Ixodes frontalis* (Panzer 1798) is a scarcely known species that is considered to be bird-specific and it has been recovered from many bird species including great tits, *Parus major* Linnaeus 1758 (Arthur, 1952; Hillyard, 1996; Tsapko, 2017). It can be found on the understory vegetation where it shares the habitat with *I. ricinus* (Heylen et al., 2014a).

Great tits are hole-breeding songbirds that are widely distributed throughout the Palearctic region. They are frequently infested with the tree-hole ticks (Arthur, 1963; Literak et al., 2007; Heylen et al., 2014c) and when foraging in the understory vegetation of forests and parks they are often exposed to *I. ricinus* and *I. frontalis* (Hubalek et al., 1996; Heylen et al., 2013, 2014c). Domestic canaries, *Serinus canaria domestica* (Linnaeus 1758), are a domesticated subspecies of the wild canary, *Serinus canaria*, a granivorous songbird living in the Macaronesian Islands and building open nests (Voigt and Leitner, 1998; Cramp et al., 1994). They do not breed or roost in cavities and therefore never come into contact with *I. arboricola*; moreover, there is no published observation of overlapping distribution between the ancestral wild canary and tree-hole ticks. It is therefore a completely novel host for *I. arboricola.*

Experiments were performed between 2012 and 2019. The number of experimental birds and the number of ticks administered varied between experiments according to trapping success of birds, availability of cages and number of ticks available for infestation. Great tits were captured from the wild within 25 km of the city centre of Antwerp (Belgium), and kept individually in cages ($80 \times 40 \times 40$ cm). Food and water were provided ad libitum. Canaries were selected from a laboratory-based population kept indoors in single-sex aviaries at a room temperature of 19–24 °C and under artificial light. Before infestation, each bird was given at least 48 h to acclimatise. In total, 66 great tits (39 males and 27 females; five birds were used twice) were infested with larvae or nymphs of *I. arboricola*, *I. frontalis* or *I. ricinus*. Twelve canaries (four males and eight females) were infested with *I. arboricola* nymphs.

Ixodes arboricola ticks came from a laboratory colony established in 2008 with the addition of wild individuals in 2017. They were fed on great and blue tits, *Cyanistes caeruleus* (for further

Table 1
Literature review on attachment sites reported for ticks on birds. We only included studies where the entire bodies of wild birds were screened. The number of infested bird individuals and the number of ticks found are given in parentheses.

Tick species (n° ticks)	Host family (n° infested birds)	Attachment site	Region (country)	Reference
Ixodes ricinus (218)	Phasianidae (>18[a])	Head	Scotland, South England (UK)	Elston et al., 2001; Hoodless et al., 2003
Ixodes ricinus (2493) *Ixodes* spp. likely *I. ricinus* (1588)	Turdidae (446) Muscicapidae (91) Sylviidae (88) Paridae (41) Acrocephalidae (66) Prunellidae (29) Fringillidae (36) Phylloscopidae (15) Troglodytidae (12) Motacillidae (10)	Head	Six federal states (Germany), Burgundy (France)	Gregoire et al., 2002; Klaus et al. 2016
Amblyomma aureolatum (699) *Ixodes auritulus* (18)	Parulidae (7) Conopophagidae (1) Furnariidae (10) Thraupidae (7) Thamnophilidae (5) Troglodytidae (7) Turdidae (104) Passerellidae (1)	Head and throat	Paraná (Brazil)	Arzua et al., 2003
Ixodes brunneus (na)	Bombycillidae (3) Fringillidae (29) Turdidae (2) Corvidae (1) Icteridae (2) Passerellidae (3) Columbidae (1)	Head, neck	Georgia, Arkansas, Tennessee, Virginia, North Carolina (USA)	Luttrell et al., 1996
Haemaphysalis leporispalustris (1171) *Ixodes scapularis* (13)	Passerellidae (157) Turdidae (204) Parulidae (29) Icteridae (2) Corvidae (2) Certhiidae (1) Mimidae (1) Fringillidae (2) Cardinalidae (1) Troglodytidae (1)	Head	Wisconsin (USA)	Nicholls and Callister, 1996
Amblyomma nodosum (17) *Amblyomma calcaratum* (11) *Amblyomma longirostre* (22) *Amblyomma maculatum/triste* (2) *Haemaphysalis leporispalustris* (1) *Haemaphysalis juxtakochi* (38)	Turdidae (12) Cardinalidae (8) Parulidae (9) Vireonidae (2) Icteridae (1) Tyrannidae (1) Passerellidae (2)	Head (78%), cloaca (10%), rest of the body (12%)	Louisiana (USA)	Mukherjee et al., 2014
Ixodes lividus (40) *Ixodes arboricola* (819)	Hirundinidae (27) Paridae (98) Sittidae (1) Muscicapidae (1) Passeridae (2)	Head	Lower Saxony, North Rhine-Westphalia (Germany)	Walter et al., 1979; Hudde and Walter, 1988
Likely *Ixodes hirsti* (na)	Meliphagidae (na)	Head	South Australia	Kleindorfer et al., 2006

(*continued on next page*)

Table 1 (*continued*)

Tick species (n° ticks)	Host family (n° infested birds)	Attachment site	Region (country)	Reference
Ixodes spp. (116)	Laridae (3) Spheniscidae (2)	Head, neck, chest (to a lower extent)	New Zealand	Heath, 2006
Ixodes uriae (~7012)	Alcidae (88) Laridae (28) Phalacrocoracidae (4)	Foot webs, back, breast, belly, crissum, tail (alcidae) Wings (50%), other body parts (50%, laridae) Wings (35%[b]), back (30%[b], phalacrocoracidae)	Alaska (USA), British Columbia (Canada)	Choe and Kim, 1987, 1988; Morbey, 1996
Ixodes signatus (224)		Head (73%[b], alcidae) Head (60%[b], laridae) Head (58%[b], phalacrocoracidae)		
Ixodes uriae (296)	Laridae (~195)	Thighs, legs, foot webs, belly, cloaca, wings, head and neck	Scotland, England (UK)	Danchin, 1992; Barton et al., 1995
Ixodes uriae (~10800)	Spheniscidae (3)	Head (adults, nymphs); lower body parts (larvae)	Crozet archipelago (French Southern and Antarctic lands)	Gauthier-Clerc et al., 1998

na, data not available.
[a] Number of infested individuals not available for Hoodless et al. (2003).
[b] Site of attachment for the remaining percentage of ticks is not stated.

details see Heylen and Matthysen, 2010). Larvae of *I. frontalis* were obtained from one engorged female while *I. ricinus* nymphs were the second generation from adults collected near Berlin, Germany, in 2014, and bred on Gerbils (*Meriones unguiculatus*) (purchased from the company Insect Services GmbH, Berlin, Germany). All ticks were kept under similar abiotic conditions (relative humidity >84%; temperature range 15–20 °C). *Ixodes arboricola* was kept in the dark, while *I. frontalis* and *I. ricinus* were kept under a 18:6h light:dark cycle.

2.2. Tick exposure procedure

Nymphs were placed on a bird's skin using tweezers, while, due to their small body size, larvae were transferred from vials to the skin using a paintbrush. We successfully used this technique previously (Heylen and Matthysen, 2010; Heylen et al., 2014b, 2017). The head part was defined as the area on top of the body between the beak and the ears (included) while the back and belly were, respectively, the dorsal and ventral areas delimited by the junction between the humerus and the scapula on one side, and the tail on the other side. Immediately after exposure, every bird was put into one or more air-permeable cotton bags, depending on the type of experiment (see Sections 2.2.1 and 2.2.2). The outer bag (10 × 20 cm) was tightly closed so that the ticks could not escape. After 90 min, the bird was gently removed from the bag and released into its cage. Cotton bags were inspected and any ticks found in a bag were counted and killed in ethanol (80%). In virtually all cases, the ticks were not damaged. The body part of tick attachment was checked by inspecting birds for feeding ticks 60–72 h after tick exposure. We thoroughly screened the birds' skin by blowing and brushing the feathers apart with tweezers (Heylen et al., 2009, 2014c). We classified ticks into four categories (Fig. 1). Ticks placed on the bird '*Ta*' were either found inside a cotton bag '*Tb*', or assumed to be infesting the bird '*Ti*'. At inspection 60–72 h later, ticks on the bird (Ti) were further subdivided into ticks engorging '*Te*' and separately counted for each body part (*Te*′) or missing '*Tm*'. The following ratios were calculated per bird individual for each experimental condition and tick × life stage combination: ticks on the bird after 90 min (Ti/Ta), ticks not infesting the bird after 90 min (Tb/Ta), ticks missing (likely due to grooming) after 60–72 h (Tm/Ti), overall infestation success (Te/Ta), and body part preference (Te′/Te).

2.2.1. Experiment 1: Tick exposure on great tits without grooming restrictions

This experiment was carried out to investigate the abovementioned ratios when hosts were not prevented from grooming and tick movements on the host body were not constrained. The final tick attachment success (Te/Ta) and tick distribution on the bird's body is therefore the result of tick movement, attachment preference and host behaviour. Tick exposure success after 90 min (Ti/Ta) needs to be interpreted in the presence of host grooming efforts. Fifteen great tits received approximately 120 *I. arboricola* larvae each, which were placed on one of the three body parts: head, belly or back (five birds in each treatment). Twenty-eight birds received 15 *I. arboricola* nymphs each (20 birds were infested on the head, four on the belly, four on the back) (see Table 2 for an overview).

2.2.2. Experiment 2: Tick exposure on great tits with grooming restrictions

This experiment tested whether ticks differ in attachment success when applied to different body parts while preventing the host from grooming. Each bird individual received ticks on the head, belly or back, depending on the treatment. After placing the ticks on the host body, the bird was immobilized to avoid preening with the beak or grooming with claws and beak.

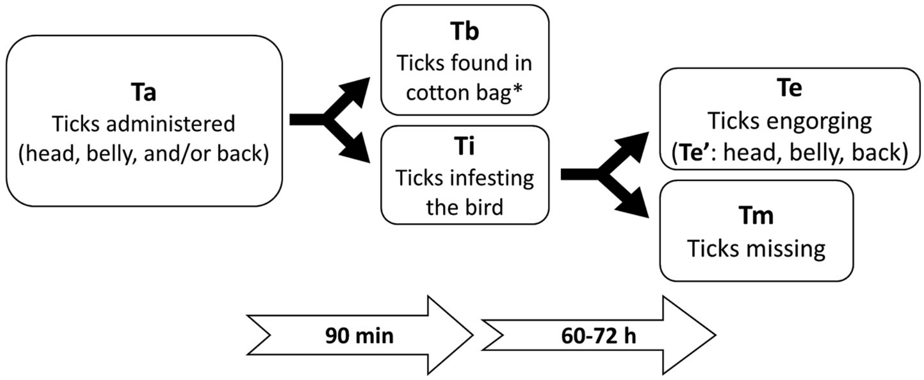

Fig. 1. Overview of the experimental workflow and of the variables considered. After 90 min, ticks placed on birds (Ta) are assigned to one of the following categories: Tb, ticks found inside a cotton bag; or Ti, ticks infesting the bird's body (i.e. Ta-Tb), and thus potentially attached and feeding. At inspection 60–72 h later, Ti are subdivided into either Te (ticks engorging on the bird) or Tm (ticks that are missing, i.e. Ti-Te). *Ticks were released in a situation either with host grooming restrictions and tick movement limitation (i.e. closed small cotton bag with head and legs protruding, and legs were tied), or without.

Table 2
Infestation scheme and number of ticks found attached to the head, belly and back for all experiments (see Sections 2.2.1 and 2.2.2 for details). Percentages of ticks found attached to the head (%) represent the ratios of the ticks eventually attached to the head with respect to the ticks initially placed on the head. Values higher than 100% highlight that ticks initially placed on other body parts (belly or back) moved to, and successfully attached to, the head region. Total percentages represent the ratios of ticks attached to any body part considering all the ticks placed on the birds.

			Attachment site			
			Head (%)	Belly	Back	Total (%)
No grooming restrictions						
Exp. 1	*Ixodes arboricola* larvae	N° great tits	5	5	5	**15**
	Ticks/bird ≈ 120; Total = 1800	N° ticks recovered	1305 (218%)	1	0	**1306 (73%)**
	I. arboricola nymphs	N° great tits	20	4	4	**28**
	Ticks/bird = 15; Total = 420	N° ticks recovered	277 (92%)	0	0	**277 (66%)**
Exp. 4	*I. arboricola* nymphs	N° canaries	4	0	0	**4**
	Ticks/bird = 12; Total = 48	N° ticks recovered	33 (69%)	0	0	**33 (69%)**
Exp. 3	*Ixodes frontalis* larvae	N° great tits	1[a]	1[a]	1[a]	**1**
	Ticks/bird ≈ 120; Total = 120	N° ticks recovered	63 (158%)	0	0	**63 (53%)**
With grooming restrictions						
Exp. 2	*Ixodes ricinus* nymphs	N° great tits	6	3	3	**12**
	Ticks/bird = 15; Total = 180	N° ticks recovered	76 (84%)	3	2	**81 (45%)**
	I. frontalis larvae	N° great tits	1	1	1	**3**
	Ticks/bird ≈ 80; Total = 240	N° ticks recovered	63 (79%)	0	0	**63 (26%)**
	I. arboricola nymphs	N° great tits	12[b]	12[b]	12[b]	**12**
	Ticks/bird = 30; Total = 360	N° ticks recovered	152 (127%)	0	0	**152 (42%)**
Exp. 4	*I. arboricola* nymphs	N° canaries	2	3	3	**8**
	Ticks/bird = 12; Total = 96	N° ticks recovered	43 (179%)	0	0	**43 (45%)**
		Total birds	**51**	**29**	**29**	**78**[c]
		Total ticks recovered	**2012 (155%)**	**4**	**2**	**2018 (62%)**

Exp., Experiment.
[a] One bird infested with 40 larvae on each body part.
[b] Twelve birds in total, each infested with 10 nymphs on each body part.
[c] Five great tits were used twice; 26 birds were infested with ticks on more than one body part.

Specifically, we inserted the bird into a small cotton bag that perfectly fitted the body and which was subsequently clamped with a plastic barrette close to the neck so that the ticks that were placed on back or belly were partially hindered in moving towards the head and vice versa. The head was kept outside the small bag. Legs were also placed outside this small bag, through small holes. Both legs were held together with a cotton elastic hair band. The bird was then placed (horizontally) into a larger outer cotton bag that was tightly closed. After 90 min the two bags were removed and inspected. This setup made it possible to separately count ticks infesting each body part in the absence of grooming (Ti/Ta) after 90 min. As the bags were removed after 90 min, '*Ti*' ticks were subsequently free to move, and resulting parts of attachment (60–72 h later) could differ from the parts where the ticks had been placed initially.

For practical reasons related to the number of birds and ticks available, we performed slightly different variations of the experiment for different tick species. Twelve birds were infested with 15 *I. ricinus* nymphs each on one body part only (six birds on the head, three on the belly, and three on the back). Similarly, three birds were infested with approximately 80 *I. frontalis* larvae (one bird for each treatment: head, belly, and back). For *I. arboricola*, 12 great tits were infested with 30 nymphs each divided over three body parts: 10 on the head, 10 on the belly, and 10 on the back (Table 2).

2.2.3. Experiments 3 and 4

We performed two additional small-scale experiments. In Experiment 3 we placed in total approximately 120 *I. frontalis* larvae (40 on the head, 40 on the belly, and 40 on the back) on a single great tit whose grooming was restricted (see Section 2.2.2). In experiment 4 we used canaries to test tick attachment success on a novel songbird species. Twelve canaries were infested with 12 *I. arboricola* nymphs each. Four canaries – exposed without grooming restrictions (see Section 2.2.1) – were infested on the head while eight canaries – exposed with grooming restrictions – were infested with ticks on head (n = 2), belly (n = 3), or back (n = 3).

2.3. Statistical analysis

Results were analysed using Generalized Linear Models (GLMs) with binomial distribution (logit link). The proportion of ticks that did not infest the experimentally exposed body part after 90 min (Tb) and the proportion of ticks feeding 60–72 h after exposure (Te) were set as dependent variables in the models. These will be referred to as, respectively, failure to attach (Tb/Ta) and attachment success (Te/Ta) (note that these do not sum to one, due to ticks that were missing). Treatment (head, belly, back), tick species and life stage were set as independent categorical variables. The latter two factors were grouped in one variable called "tick batch" (AL: *I. arboricola* larvae, AN: *I. arboricola* nymphs, RN: *I. ricinus* nymphs, FL: *I. frontalis* larvae). Separate models for each experiment were run to analyse the two dependent variables as defined above. Differences in attachment success between birds exposed to *I. frontalis* larvae were not statistically analysed due to the very small sample size. Tick batch was not included in the model for experiment 2 since birds infested with *I. arboricola* and *I. ricinus* nymphs were analysed separately. The proportion of *I. arboricola* nymphs that failed to attach in experiment 2 (i.e. Tb/Ta) was analysed through a Chi-squared test. To test if the attachment success to a specific body part differed with respect to the exposed body part we ran a GLM for each experiment with treatment as an independent categorical variable and the proportion of attachment to the head as a dependent variable. The effect of tick life stage on attachment success to the head was investigated by setting treatment, batch, and their interaction as fixed effects. Two outliers were removed from experiment 1 in all relevant models since the larvae recovered exceeded the approximate number of larvae put on the bird. Likelihood ratio tests (LRTs) were used to obtain the Chi square values of the models. We used the "multcomp" package in R to calculate the pairwise comparisons (Hothorn et al., 2008). P values less than 0.05 were considered significant. Except where indicated, percentages are given with respect to the total number of ticks put on the birds for the same treatment and batch. Data analysis was performed in R v 3.5.2 (R Core Team, Vienna, Austria; 2018).

2.4. Data accessibility

Data are available in Mendeley Data, V1, doi: https://doi.org//10.17632/3bnhvydzc2.1.

3. Results

3.1. Experiment 1: Tick exposure without grooming restrictions

In total, 1306 *I. arboricola* larvae (Te, out of Ta = 1800) attached to the birds with 1305 larvae found on the birds' heads (72.5% of all larvae; Table 2) and one on a bird's belly. The attachment success to the head significantly differed between ticks exposed to the belly and back: 394 out of 600 (66%) attached to the head when put on the head while 369 out of 600 (62%), and 542 out of 600 (90%) attached to the head when put on the belly and back, respectively. Seventy-one larvae (3.9% of all larvae) were found in the cotton bag after exposure (Tb). Similarly, all 277 *I. arboricola* nymphs (Ta = 420) feeding on great tits attached to the head independently of the body part of exposure (Table 2). The attachment success to the head did not differ significantly between exposed body parts: 201 out of 300 nymphs (67%) attached when put on the head while 42 out of 60 ticks (70%), and 34 out of 60 ticks (57%) attached when put on the belly and back, respectively. Forty-two nymphs failed to attach (Tb), of which 18 when placed on the head (6%), nine (15%) on the belly, and 15 (25%) on the back (for an overview of the mean number of 'Tb' ticks see Table 3).

The proportion of ticks that successfully attached on the body part where the original exposure took place strongly differed among the treatment groups (χ^2_2 = 1235.12, P < 0.001; n = 41). Eventually, all attached nymphs were found on the head, no matter on which body part they were released. The GLM on the proportion of ticks found in the bag after exposure showed a significant interaction between treatment and tick batch (i.e. tick species and life stage) (χ^2_2 = 10.734, P = 0.005; n = 41). This result is mainly due to a higher proportion of nymphs found in the bag (Tb) when exposed to the back compared with larvae exposed to the same body part. The attachment success on the head differed signifi-

Table 3
Mean number of ticks administered per bird (Ta), infesting the bird (Ti), engorging (Te), found in cotton bags (Tb), and missing (Tm). Data shown for all experiments (Exp.): *Ixodes arboricola* larvae (AL), *Ixodes arboricola* nymphs (AN), *Ixodes ricinus* nymphs (RN), *Ixodes frontalis* larvae (FL).

Exp. (instar)	Area exposed (Ta)		Te (±S.E.M.)						Tb		Ti		Tm	
			Head		Belly		Back							
1 (AL)	Head	(~120)	79	(4)	0		0		1	(1)	119	(1)	40	(4)
	Belly	(~120)	74	(11)	0		0		9	(5)	111	(5)	37	(11)
	Back	(~120)	108	(15)	0		0		3	(1)	117	(1)	30	(8)
1 (AN)	Head	(15)	10	(1)	0		0		1	(0)	14	(0)	4	(1)
	Belly	(15)	11	(3)	0		0		2	(2)	13	(2)	2	(1)
	Back	(15)	9	(2)	0		0		4	(1)	11	(1)	3	(1)
2 (RN)	Head	(15)	12	(1)	0		0		1	(0)	14	(0)	3	(1)
	Belly	(15)	2	(1)	1	(1)	1	(1)	8	(1)	7	(1)	4	(1)
	Back	(15)	0		0		0		10	(1)	5	(1)	4	(1)
2 (FL)	Head	(80)	50		0		0		0		80		30	
	Belly	(80)	1		0		0		73		7		6	
	Back	(80)	12		0		0		64		16		4	
2 (AN)	Head, belly, back (30)		13	(1)	0		0		9	(1)	21	(1)	8	(1)
3 (FL)	Head, belly, back (120)		63		0		0		8		112		49	
4 (AN)[a]	Head	(12)	8	(1)	0		0		2	(1)	10	(1)	2	(0)
4 (AN)	Head	(12)	9	(1)	0		0		2	(2)	10	(2)	1	(1)
4 (AN)	Belly	(12)	2	(1)	0		0		8	(1)	4	(1)	2	(0)
4 (AN)	Back	(12)	6	(1)	0		0		2	(0)	10	(0)	3	(1)

[a] Four canaries without grooming restriction.

cantly with respect to treatment (χ^2_2 = 6.978, P = 0.031; n = 41) with larvae initially put on the belly having significantly lower attachment success compared with larvae put on the back (P = 0.025). Tick life stage had no effect on attachment success on the head (χ^2_1 = 0.007, P = 0.933; n = 41).

3.2. Experiment 2: Tick exposure with grooming restrictions

In the birds infested with *I. ricinus*, 81 nymphs (Ta = 180) attached in total: 76 nymphs on the birds' heads (42% of all nymphs; Table 2), three (7%) on the birds' bellies and two (4%) on the backs. Specifically, 70 out of 90 nymphs (78%) attached to the head when put on the head, while only five out of 45 (11%), and one out of 45 (2%) attached to the head when put on the belly and back, respectively. Three ticks (3%) failed to attach when put on the head, 24 (53%) on the belly, and 31 (69%) on the back. The proportion of ticks that attached to the exposed body part was significantly different between treatments (χ^2_2 = 75.7, P < 0.001; n = 12). Moreover, the proportion of ticks that failed to attach was significantly higher when ticks were put on the back or on the belly compared with ticks put on the head (both pairwise comparisons: P < 0.001). For *I. ricinus,* the attachment success to the head differed significantly with respect to treatment (χ^2_2 = 108.83, P < 0.001; n = 12), with ticks initially put on the head having significantly greater attachment success (both comparisons P < 0.001).

With regard to *I. arboricola*, all 152 feeding nymphs (Ta = 360) were attached to the head (42% of all nymphs and 127% of nymphs put on the head, see Table 2) while no nymph attached to the back or the belly. Ninety-five nymphs (40%) failed to attach when placed on the birds' backs or bellies but only 14 (6%) when placed on the heads. The proportion of ticks that failed to attach was significantly different between exposed body parts ($\chi^2_1 = 28.226$, P < 0.001; n = 12). In the three birds infested with *I. frontalis,* all 63 engorging larvae (Ta = 240; 26% of all larvae) were feeding on the head while no tick attached to the back or belly. Specifically, 50 out of 80 (63%) attached to the head when put on the head, while one out of 80 (1%) and 12 out of 80 (15%) attached to the head when put on the belly and back, respectively. Moreover, 137 out of 160 (86%) *I. frontalis* larvae failed to attach when administered on the belly or back. In contrast, no larvae were found in the bag (Tb) for the great tit infested on the head.

3.3. Experiments 3 and 4

As regards the single bird exposed to larvae of *I. frontalis* without grooming restrictions, we found 63 larvae (53% of all larvae; Table 2) feeding on the bird, all of which attached to the head. Similarly, for the four canaries infested on the head without grooming restrictions, 33 ticks (Ta = 48; 69%) attached to the head while no tick attached to the back or the belly. Only seven ticks (15%) failed to attach after exposure (Tb). When canaries were prevented from grooming, 43 ticks (Ta = 96) attached to the eight birds, again all of them on the head (Table 2): 18 out of 24 nymphs (75%) attached when put on the head while six out of 36 (17%), and 19 out of 36 (53%) attached to the head when put on belly or back, respectively. The proportion of ticks found in the bag after exposure differed between treatments (χ^2_2 = 20.611, P < 0.001; n = 8): four ticks (17%) failed to attach when put on the head compared with 23 ticks (64%) and seven ticks (19%) for the belly and back treatment, respectively. The attachment success to the head differed significantly with respect to treatment (χ^2_2 = 22.813, P < 0.001; n = 8) with ticks initially put on the belly having a significantly lower attachment success compared with ticks put on the head or back (both comparisons P < 0.006).

3.4. Combined data from all experiments

Considering all ticks put on all experimental birds, we found a total of 2018 ticks (Ta = 3264) feeding 60–72 h after exposure: 2012 ticks attached to the head, four ticks attached to the belly and two ticks to the back (Table 2, Fig. 2; ticks attached to the head of every bird – see Supplementary Table S1). On the head, most ticks were located next to the eyes and in the area between the beak and the eyes. The GLM run on the data from all tick batches – excluding *I. frontalis* due to the very small sample size and the birds infested on multiple body parts – showed a significant effect of treatment (χ^2_2 = 1416.640, P < 0.001, n = 65) on the proportion of ticks found attached to the same part where they were initially exposed. The proportion of ticks that did not infest the bird after 90 min (Tb/Ta) was significantly related to the interaction between treatment and experiment (χ^2_2 = 9.370, P < 0.01, n = 65): infestation failure was lowest for the head treatment in all experiments but the proportion of ticks that failed to attach and the treatment associated with highest attachment failure differed between batches and experiments. Specifically, ticks in experiment 2 exposed to the back and ticks in experiments 2 and 4 exposed to the belly showed a much higher infestation failure compared with the other experiments. Considering all experiments together, we found a lower infestation failure for ticks placed on the head (Fig. 3).

Overall, 25% (807 out of 3264) of the ticks placed on the birds was never found (Tm). A number of factors hindering tick detection

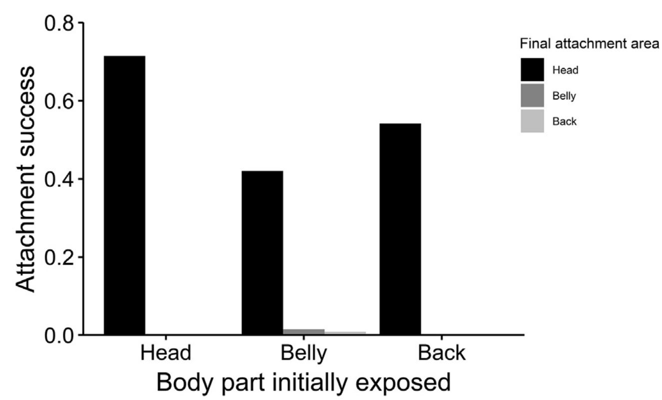

Fig. 2. Proportion of attached ticks with respect to body part where attached and body part where initially exposed. Data from all experiments were pooled, weighed based on the number of infested birds in each treatment, and averaged. The single great tit infested with *Ixodes frontalis* and the birds simultaneously infested on multiple body parts are not included.

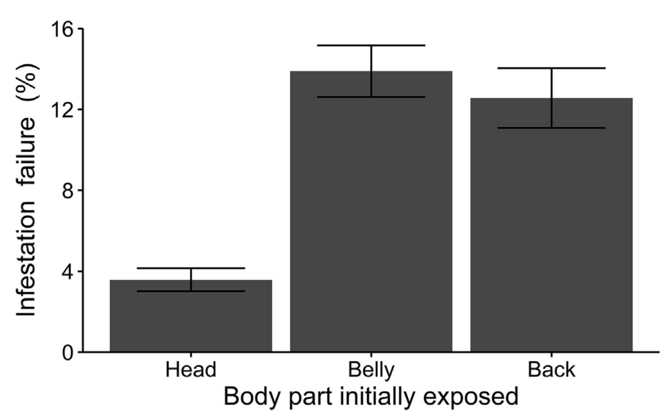

Fig. 3. Percentage of ticks found in the bag after 90 min of exposure. Data include average percentage of *Ixodes arboricola* larvae and nymphs (experiments 1 and 4), and *Ixodes ricinus* nymphs (experiment 2) that failed to attach with respect to the body part of exposure. Error bars represent ±1 standard error.

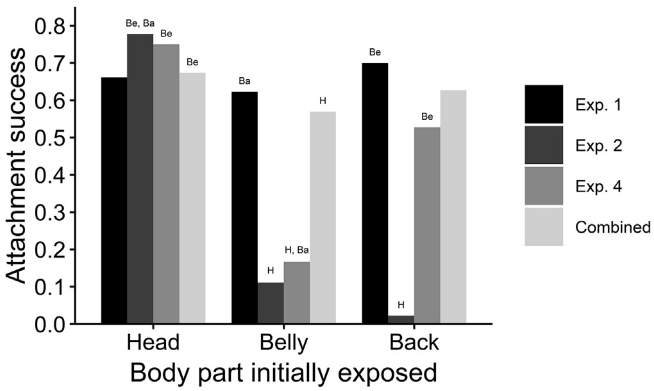

Fig. 4. Proportion of ticks attached to the head with respect to the body part initially exposed for all main experiments (exp. 1, 2, 4). Letters above bars indicate a significant difference ($P < 0.05$) with respect to head (H), belly (Be), or back (Ba) of the same group.

are worthy of mention. The small size of unfed ticks (especially larvae) and the ticks' habit of crawling at the base of the plumage are likely the most important ones. Feathers needed to be moved to locate the feeding ticks, making very accurate screening sometimes too stressful for the bird. Alternatively, ticks may have remained on the bird without attaching during the tick exposure procedure and might have been groomed away by the host during the following hours. When attachment success on the head was compared between exposed body parts considering experiment as a fixed effect (experiments 1, 2, 4), we found a significant interaction between experiment and treatment (χ^2_2 = 44.043, $P < 0.001$; $n = 61$). In particular, exposure to the head led to the highest attachment rate in all experiments but experiments 2 and 4 showed a lower attachment success on the belly and the back compared with experiment 1. When all three of these experiments were pooled together, the attachment success on the head differed significantly with respect to treatment (χ^2_2 = 19.830, $P < 0.001$; $n = 61$). Ticks initially placed on the head showed significantly higher attachment success only when compared with ticks put on the belly ($P < 0.001$, Fig. 4).

4. Discussion

We found highly similar attachment behaviour in three tick species that differ in ecology and host specificity, infesting two different host species. All ticks showed a very strong preference for attaching on the bird's head. Even when ticks were placed on other body parts under optimal conditions for attachment (i.e. in the absence of host grooming for 90 min) they failed to attach there. The vast majority of the ticks that were put on the belly or the back and eventually attached to the bird had moved to the head. We observed exactly the same attachment patterns in the domestic canaries as in the great tits, even though canaries have never been exposed to ticks before and would be a very unusual host in the wild for at least one of the main tick species used, the tree-hole tick (*I. arboricola*) which only infests hole-nesting birds. All experimental outcomes are highly congruent with the literature overview in Table 1, and suggest that the preference of *Ixodes* ticks for the songbird's head is a very robust behavioural characteristic. Similar attachment patterns have been found in other tick genera such as *Amblyomma* and *Haemaphysalis* infesting representatives of many different songbird families (Table 1).

Tick attachment to the head was higher when bird grooming was allowed, while there was lower attachment success when grooming was restricted. This outcome might suggest that grooming behaviour was not effective in reducing tick load while ticks were moving on the host body. It is worth noting that this does not imply grooming is not effective in reducing the number of ectoparasites once feeding is established. The lower attachment success when grooming was restricted points to the inner bag not only having prevented grooming but also having acted as a physical barrier to ticks moving towards other body parts, forcing on-site attachment and possibly leading to attachment rejection by the tick. Additionally, we showed that ticks can infest a host even when they do not immediately come into contact with a suitable body part, by moving along the host body.

Interestingly, *I. ricinus* shows the same infestation preference in birds as the two bird-specialised ticks, even though this generalist tick prefers very different body parts when infesting non-avian hosts such as the forelimbs of lizards (Bauwens et al., 1983), axillae and udder of cattle (L'Hostis et al., 1994), or legs of humans (Wilhelmsson et al., 2013). In light of our findings, the infestation patterns of *I. ricinus* on those other vertebrates are likely also driven by an active choice of the parasite rather than the result of host grooming activity.

Tick attachment preference can be adaptive for a number of non-mutually exclusive reasons. Primarily, preference for the head could have evolved in response to host grooming that causes severe fitness reduction in the parasite (Clayton et al., 2005; Waite et al., 2012). Ticks that are groomed away, are generally critically injured and even if they survive may have little opportunity for re-infestation. Generally, birds are very effective in reducing ectoparasites, whereby the beak – which has access to the complete body except the head – plays a very important role. It has been shown that minor beak deformities increase ectoparasite load (Cotgreave and Clayton, 1994; Clayton et al., 2010) and that parasites act as a selective pressure on beak shape (Villa et al., 2018). Since most songbirds do not show allo-preening, the head is a safe place for the tick to attach as it cannot be groomed via the beak, and it is difficult to be reached by claws. Hence, we believe that preening avoidance is the first proximate causation for the pattern observed. Interestingly, more scattered infestation patterns have been found for *I. uriae*, a generalist hard tick feeding on seabirds (Arthur, 1963; Dietrich et al., 2014). *Ixodes uriae* can be found attached to different body parts such as the lower body parts, depending on the host species and tick life stage (Table 1). Seabirds differ from passerine species in several aspects of ecology and morphology which might explain the difference in the pattern of tick attachment. In addition, the different shape of the beak and the webbed claws might result in reduced grooming efficiency, thus reducing the selective pressure acting on attachment sites. Unfortunately, to the best of our knowledge no data on grooming efficiency in marine birds is available to investigate this hypothesis. Also, several aspects in the life history of *I. uriae* differ from other ixodid ticks (McCoy and Tirard, 2002).

Alternative explanations may be related to feather morphology and vascularization of the head versus other body parts. Feathers of the head are shorter and closer to each other compared with other body parts (Ammann, 1937; Markus, 1963; Deville et al., 2014; Mathewson et al., 2018; Strubbe, 2019), possibly providing a slightly different and more suitable microclimate for ticks. Here, these hematophagous parasites might find a different and more stable microclimate facilitating water balance (Sauer and Hair, 1971; Stafford, 1994) and thus permitting faster engorgement. Support for this hypothesis is given by the longer feeding duration of *I. uriae* nymphs attached to unfeathered body parts compared with feathered ones (Barton et al., 1995).

Experiments with different ixodid species show that when ticks engorge on body parts with scarce blood supply, feeding takes slightly longer and leads to a reduced weight of engorgement (Balashov, 1972). Compared with other body parts, the bird's head is a well vascularised area covered by a thin skin layer (Pass, 1995; Stettenheim, 2000). This might facilitate tick attachment and fast and effective engorgement. In particular, the orbit, the oral cavity and the nasal cavity are areas of thermal exchange supplied by a main source of blood splitting into numerous blood vessels (Porter and Witmer, 2016). Also, a higher feather density might protect ticks from airflow while the bird is in flight (Choe and Kim, 1991). Hence, choosing the head might reduce feeding time (and its associated risks), protect against host grooming and directly increase tick fitness. Tick fecundity is in fact correlated to weight after engorgement (Gray, 1981).

In addition, aggregation benefits could potentially reinforce the observed attachment preference. First, tick aggregation might facilitate feeding success or increase engorgement weight as, respectively, shown by experiments on *I. arboricola* (Van Oosten et al., 2016) and *I. ricinus* (Ogden et al., 2002). Similarly, moulting success was positively correlated to the density of *Ixodes scapularis* larvae engorging on naïve hosts (Hazler and Ostfeld, 1995). This in itself might not be sufficient to explain such a strong choice for a specific body part, but could enhance the preference once it is established.

When looking at these findings from the perspective of disease ecology, the clear preference for the head increases the chances of transmission of tick-borne pathogens while co-feeding on the same host (Ogden et al., 1997; Voordouw, 2015) since ticks are vectors of a large number of infectious pathogens transmitted to humans and other animals (Labuda and Nuttall, 2004; Goodman et al., 2005; Sonenshine and Roe, 2013; Baneth, 2014). A very direct way for the spread of diseases can occur through co-feeding transmission that takes place when ticks feed in close proximity in time and space (Randolph et al., 1996; Ogden et al., 1997; Randolph and Gern, 2003; Randolph, 2011). Pathogens that remain in the area of inoculation before disseminating all over the body can be ingested with the blood meal by ticks feeding in the infected area (Shih et al., 1992; Labuda et al., 1993; Gern and Rais, 1996). Hence, different tick species feeding on the same body part can enhance the transmission rate of pathogens within and between tick and host species.

In conclusion, our results show that different species of ixodid ticks have a consistent preference for attaching on the head of their songbird hosts, and move to the head even if placed on other body parts. This experimental finding is consistent with literature reports on several tick species on songbirds and other terrestrial birds. Hence, we suggest the existence of a consistent pattern of attachment across ixodid species with the exception of *I. uriae* on seabirds. Similar patterns of attachment might also occur in different host taxa and have an impact on tick survival and evolution, as well as on disease transmission. We hope future studies will help to address this question.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.09.003.

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