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ORIGINAL PAPER

An experimental test to compare potential and realised specificity in ticks with different ecologies

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Abstract The majority of studies on ecological specialisation rely on data reflecting realised specificity, without considering species' potential specificity. Most species of ticks, a large family of hematophagous ectoparasites, have a narrow host range in nature, but it is unclear whether this is due to host-driven adaptations or other processes (such as off-host abiotic environment). We investigated the potential specificity of two tick species with contrasting ecology by infesting three avian host species that occur in the same off-host macrohabitat but are unequally infested by the ticks in nature (i.e. have contrasting realised specificity). The endophilic specialist tick *Ixodes arboricola* resides inside the hosts' nest and has high realised host specificity, whereas the exophilic generalist tick *I. ricinus* encounters hosts in the field and has very low realised specificity. As hosts, we used great tits (frequently infested by both tick species), blackbirds (frequently infested by *I. ricinus* but never by *I. arboricola*) and great spotted woodpeckers (no ticks of either species have been reported). If realised specificity is constrained by host-driven adaptations there should be no differences between potential and realised specificity, whereas if realised specificity is constrained by other processes potential specificity and realised specificity should be different. We found that attachment rates and weight during feeding of *I. arboricola* were lower on blackbirds than on great tits, whereas there were no such differences for *I. ricinus*. No ticks of either species attached to woodpeckers. These results indicate that realised host specificity of ticks is, at least partially, constrained by host-driven adaptations. This specificity therefore strongly depends on the ticks' encounter rates with particular host types, which are affected by the ticks' off-host ecological requirements, behaviour and life-history characteristics.

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Introduction

The evolution of specialisation has long been of interest to evolutionary ecologists (Euzet and Combes 1980; Futuyma and Moreno 1988; Kassen 2002; Poisot et al. 2011; McCoy et al. 2013). Specificity, which is the result of specialisation, arises because increased performance in some habitats is associated with decreased performance in others due to trade-offs or constraints (Kassen 2002). Similar to the concept of the niche (Hutchinson 1957), specificity of an organism can be broken down into two distinct concepts. The full range of habitats usable by an organism defines its potential specificity, while the observed use of these habitats is its realised specificity. Potential specificity of an organism is the result of its intrinsic physiological limits that allow it to establish, maintain itself and reproduce within a specific range of habitats (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). In contrast, realised specificity is primarily constrained by the ability to reach usable habitats (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). From an evolutionary point of view, potential specificity summarises the degree of adaptation to a specific range of habitats and is determined by evolutionary interactions between genotype and habitat, whereas realised specificity reflects the impact of ecology, chance events and history on potential specificity (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). In non-parasitic organisms, realised specificity is generally summarised in terms of dispersal limitation (Poisot et al. 2011). In parasitic organisms, it is assumed more complex and includes spatial and temporal overlap between parasites and hosts and parasite's ability to locate, invade and exploit potential hosts (Poulin and Keeney 2008; McCoy et al. 2013).

For parasitic organisms, the degree of specificity affects both its population dynamics and its evolutionary trajectory. Specificity of parasites has often been reduced to specificity towards hosts (i.e. host specificity) because host specificity is arguably the most fundamental property of parasitic organisms (Poulin and Keeney 2008). Traditionally, host specificity has been quantified as the number of host species used under natural circumstances, and in this context most parasites are considered highly host-specific (Poulin and Keeney 2008). However, such high host specificity may not just reflect which host species are compatible but also which are encountered, because typically fewer hosts are encountered than that could potentially be compatible. Therefore the possibility to encounter a host species and host compatibility may act as 'filters' on the evolution of host specificity (Euzet and Combes 1980; Combes 2001). First, the parasite must pass through an encounter filter to reach a potential host. If successful, it must also pass through a compatibility filter, determining whether the parasite can circumvent or disable host defences and whether the host is nutritionally compatible for parasite development. For ectoparasites, potential specificity should be determined by host and off-host habitat and not just by the host as in endoparasites (Klompen et al. 1996).

If a parasite species frequently encounters the host species (encounter filter open) and can feed successfully on that host species (compatibility filter open), the host species is part of the parasite's realised specificity. But if parasites do not occur on a particular host species in nature there are three mutually exclusive scenarios. First, a parasite species may rarely or never encounter a particular host species (encounter filter closed) but have the potential to feed successfully on that host species (compatibility filter open). In this case, realised specificity and potential specificity are different. Second, a parasite species may frequently encounter a specific host species (encounter filter open) but be unable to feed successfully on that host species (compatibility filter closed). In this case, potential and realised specificity are equivalent. Finally, a parasite species may rarely or never encounter a certain host species (encounter filter closed) but even if there were encounters the parasite would be unable to feed successfully on that host species (compatibility filter closed). In this case, too, realised and potential specificity are equivalent. Which scenario is the case can only be unravelled through experimental testing.

An increasing number of studies on ectoparasites have opened the encounter filter experimentally to investigate the compatibility of novel hosts, thus comparing realised and potential specificity (for a review, see Poulin and Keeney 2008). If potential and realised specificity are equivalent, compatibility of novel and natural hosts should be different, whereas if potential specificity is lower than realised specificity, compatibility of novel and natural hosts should be equivalent. In the majority of studies natural hosts are more compatible than novel hosts (e.g. Tompkins and Clayton 1999; Giorgi et al. 2004; Esbérard et al. 2005; Goddard et al. 2005; Glennon et al. 2007; Kuris et al. 2007). This suggests that potential specificity is similar to realised specificity because host-driven adaptations are the principle drivers of parasite realised specificity. It also suggests that potential host specificity, which is measurable under laboratory conditions, is a good predictor of parasite realised specificity.

The superfamily of ticks (Ixodoidea) encompasses approximately 800 species of hematophagous ectoparasites, and realised specificity of most tick species is limited to one or only a limited number of host species (Hoogstraal and Aeschlimann 1982). However, a strong positive correlation was found between the degree of host specificity and sampling effort, and host range might be broader than currently quantified (Klompen et al. 1996; Cumming 1999; Nava and Guglielmone 2013). High potential host specificity was initially expected because various structural modifications of tick mouthparts and legs are associated with particular host species (Hoogstraal and Kim 1985) and because the complexity of the host's immunological and behavioural responses requires host-specific circumventive mechanisms (Magalhães et al. 2007). Because related species are often more similar in terms of immunology, physiology and morphology, host phylogeny was attributed a considerable role in the evolution of tick host specificity (Hoogstraal and Aeschlimann 1982; Hoogstraal and Kim 1985). However, meta-analyses on realised specificity show weak support for hypotheses that tick evolution arose through host adaptation, host specificity or cospeciation, and it has been argued that realised specificity is explained much better by biogeography, host ecological similarities and abiotic conditions during the long off-host period (Klompen et al. 1996; Nava and Guglielmone 2013). Although a limited number of experimental studies suggest that ticks generally perform better on natural than novel hosts (Slowik and Lane 2009; Brunner et al. 2011; Harrison et al. 2012; Dietrich et al. 2014), more investigations are required to assess the importance of host-driven adaptations for realised host specificity in ticks.

In order to evaluate the importance of potential host specificity for realised host specificity, we used a laboratory experiment to investigate whether potential host specificity of two sympatric tick species corresponds to their realised host specificity. *Ixodes arboricola* Schulze & Schlottke 1929 is a specialist endophilic tick and its realised specificity almost exclusively consists of cavity-nesting birds, predominantly great and blue tits (*Parus major* L., *Cyanistes caeruleus* L.) (Walter et al. 1979; Petney et al. 2011; Heylen et al. 2014; Van Oosten et al. 2014b). Endophilic ticks remain hidden inside or near to their hosts' nests or burrows and only attach to the host when it arrives (Hoogstraal and Aeschlimann 1982; Sonenshine 1991; Hillyard 1996). Whereas this maximises the possibility of finding a host, nests are visited only by a limited variety of host species and realised host specificity of endophilic ticks is high (Petney et al. 2011; Gray et al. 2014). *Ixodes ricinus* L., on the other hand, is a typical generalist exophilic tick that has been recorded on a wide range of vertebrate hosts, and can be found in the understorey of forests and parks (Gray 1991; Hillyard 1996; Gern 2005; Marsot et al. 2012; Schulz et al. 2014). Exophilic ticks typically seek hosts actively by climbing up the vegetation and waiting for them to pass (Hillyard 1996). Therefore exophilic ticks may encounter many different host species and, indeed, realised specificity is typically lower than for endophilic ticks (Hoogstraal and Aeschlimann 1982; Hillyard 1996).

As hosts, we used three forest birds that are common in the macrohabitat of both tick species, but for which the state of the encounter filter and thus realised specificity vary due to their contrasting habitat use. The great tit regularly feeds on the ground and in low vegetation, and nests and roosts in extant cavities (Gosler 1993). Therefore the encounter filter is open for both *I. ricinus* and *I. arboricola*. The blackbird (*Turdus merula* L.) commonly feeds on the ground and in low vegetation but never nests in cavities (Cramp 1988). Thus, the encounter filter is open only for *I. ricinus*. The great spotted woodpecker (*Dendrocopos major* L.) rarely feeds on the ground but nests and roosts in cavities (Cramp 1985). However, woodpeckers are primary cavity nesters and therefore tend to excavate new cavities rather than using extant ones. Therefore the encounter filter is open for either tick species, but only to a limited extent due to limited habitat overlap between woodpeckers and ticks.

We had two contrasting hypotheses. On the one hand, realised specificity might be constrained by host-driven adaptations. In this scenario, experimentally measured potential specificity is equivalent to realised specificity observed in nature, and performance on novel hosts is different from natural hosts. For *I. arboricola*, we would expect attachment success and engorgement weight on great tits to be different from blackbirds and woodpeckers, and for *I. ricinus* we would expect attachment success and engorgement weight on great tits and blackbirds to be similar, but different on woodpeckers. On the other hand, realised specificity might be constrained only by other processes, such as habitat adaptation, behaviour and life-history characteristics, that affect the encounter filter but not host compatibility. In this scenario, potential specificity is much lower than realised specificity, and performance on novel and natural hosts is equivalent. This would be reflected by equivalent attachment success and engorgement weights of both ticks on all bird species.

Materials and methods

Study location

Experimental infestations took place at our laboratory and aviary at Campus Groenenborger in Antwerp, Belgium in 2014. *Ixodes arboricola* came from a laboratory stock that has been established in 2007 with ticks from nest boxes used by great and blue tits in woodland areas near Antwerp (mainly Peerdsbos, Brasschaat). All *I. arboricola* nymphs used in the current experiment fed on great tits in previous experiments. *Ixodes ricinus* were collected up to 5 days before infestation in Grenspark de Zoom, Essen, by dragging a white flannel flag over suitable vegetation. Birds were captured at Peerdsbos, Brasschaat in

June and July 2014 (20 woodpeckers and 19 great tits) and, in September 2014, at Kalmthoutse Heide, Kalmthout (21 blackbirds and 2 great tits) and Campus Groenenborger, Antwerp (20 great tits). Birds without a metal ring received one for identification. Birds used for previous infestations were released. Each bird was inspected for ticks immediately after capture, and any attached ticks were removed with tweezers for identification. All birds were housed in individual cages (8 m^3) in an aviary at Campus Groenenborger. All cages were supplied with tree trunks as climbing or perching substrates and birds had constant access to water. Woodpeckers and great tits received a diet of mealworms, fat balls, peanuts and sunflower seeds ad libitum. Blackbirds received a diet of mealworms, insect pate, dried bread and apples ad libitum. Cages for woodpeckers and great tits were provided with a nest box (3.4 dm^3 for great tits, 42.9 dm^3 for woodpeckers).

Ticks—*Ixodes arboricola*, *Ixodes ricinus*

All ixodid ticks go through three active stages (larva, nymph, adult) and during each stage, with the exception of adult males, take a single blood meal either to moult (larvae, nymphs) or to reproduce (Sonenshine 1991). *Ixodes arboricola* is an endophilic tick, with the entire life cycle restricted to natural and man-made cavities (Liebisch 1996). It is widely distributed over Europe (Liebisch 1996; Petney et al. 2011). The tick shows adaptive behavioural mechanisms to ensure detachment in suitable habitat, such that it detaches predominantly at night, when its hosts roost in cavities (Heylen and Matthysen 2010), and delays detachment when hosts roost outside (White et al. 2012). As such, its realised specificity only includes hosts that make use of such cavities (Walter et al. 1979; Heylen et al. 2014), and this limits its dispersal capabilities between cavities (Van Oosten et al. 2014a).

Ixodes ricinus is an exophilic generalist tick found in deciduous woodland, meadows and moorland across Europe, Russia, North Africa and the Middle East (Gern 2005; Petney et al. 2011). It climbs up to some vantage point in the lower vegetation ("questing") from where it contacts passing vertebrate hosts. To ensure detachment in the field rather than a cavity when infesting hosts that make use of cavities, it detaches predominantly during the day (Heylen and Matthysen 2010 and references therein). Immature life stages infest a wide array of terrestrial vertebrates, including songbirds, although adults are only found on larger animals (Gray 1991; Gern 2005).

Avian hosts—great tit, blackbird, great spotted woodpecker

The great tit (family Paridae), the commonest secondary cavity-nesting bird in Western Europe, uses yet available natural or artificial cavities for breeding and roosting (Gosler 1993) and is therefore frequently exposed to *I. arboricola* (Literák et al. 2007; Heylen et al. 2014). The foraging niche includes the lower vegetation strata inside forests, resulting in frequent *I. ricinus* infestations, especially during the breeding season (Heylen et al. 2014).

The common blackbird (family Turdidae) is one of the most common birds in Europe, breeding in any habitat with woody cover at high densities (Cramp 1988). Blackbirds build open nests in the vegetation or occasionally on the ground (Cramp 1988), where they never encounter *I. arboricola* (Literák et al. 2007; Norte et al. 2012). Because they forage on the ground and lower vegetation strata, they are among the most heavily infested hosts for *I. ricinus* (Marsot et al. 2012; Norte et al. 2012).

The great spotted woodpecker (family Picidae), a widespread breeding bird in Europe mostly found in mature forests, usually excavates new cavities for breeding and roosting

(Cramp 1985). Generally woodpeckers do not forage on the ground but they may do this in fall and winter to collect seeds and nuts (Cramp 1985). So far, no ticks have been reported on great spotted woodpeckers (Literák et al. 2007; Marsot et al. 2012; Norte et al. 2012). In our own capture sessions in Peerdsbos, Brasschaat, 101 great spotted woodpeckers were inspected for ticks over a five-year period, but ticks were never found, whereas great tits were frequently infested with either tick species (unpublished results).

Experimental infestations

We performed standardised infestation experiments with both tick species on each of the three avian hosts. Because of constraints in housing facilities and seasonal variation in capture success of the different host species, two separate experiments were performed in a parallel group design, in which similar numbers of individuals of two host species were infested. The most common host (great tit) was used in both experiments.

Birds were infested within a day after capture. Each bird was infested with ten nymphs of either *I. arboricola* or *I. ricinus*, which were placed under the feathers on the occipital side of the head with a small brush. Birds were placed individually in a cotton bag (15 × 25 cm for great tits, 25 × 40 cm for woodpeckers and blackbirds) for 1 h. Afterwards, the bird was returned to its cage. The birds were not inspected for attached ticks at this moment since unfed ticks are difficult to detect without prolonged manipulation, which may disturb the attachment process (Heylen and Matthysen 2011). Rather, birds were inspected for attached ticks 48 h after infestation by lifting the feathers with tweezers. Ticks were counted and removed and subsequently weighed. The birds were released the same day.

Statistical analyses

All data analyses were done in R v 3.1.1 (R Core Team 2013). For each tick species, we used two separate linear mixed-effects models from package LME4 v 1.1-7 (Bates et al. 2015) to evaluate whether attachment success after 48 h (i.e. the proportion of ticks attached to individual hosts; logit-link, binomially distributed residuals) and engorgement weight (identity-link, normally distributed residuals) of the nymphs differed between tick species and among bird species. Engorgement weight was standardised among bird species within tick species to obtain relative feeding performance across bird species. All models considered individual ticks as the replicated unit, and by adding bird identity as a random effect nested within infestation session we corrected for the non-independence in the response variables of the ticks attached to an individual bird.

Fixed effects in all models included: bird species (blackbird and great tit; woodpecker in the model for attachment success) and the number of ticks found on birds pre-experimentally (log-transformed; Table 1). In all models a stepwise selection procedure was used in which the model was iteratively refitted after exclusion of the least significant fixed effect. Post hoc tests were conducted for the main effect bird species with package multcomp v 1.4-0, which corrects for multiple comparisons with Tukey Contrasts (Hothorn et al. 2008). Because attachment success across all woodpeckers was zero, variance estimates could not be calculated and the models did not converge. This was resolved by using a dummy variable, in which attachment of a single nymph of both tick species was changed to “success”, hence introducing artificial variance. This had no effect on the results due to the large sample size (*I. ricinus* $N = 100$; *I. arboricola* $N = 90$). To fulfil normality

Table 1 Pre-experimental infestation intensity and number of ticks ± SE on infested birds per instar (larva, nymph, adult) of *I. ricinus*, *I. arboricola* and *I. frontalis* on great tits, blackbirds and woodpeckers

	Great tit	Blackbird	Woodpecker
I. ricinus			
L	7 %; 2.3 ± 1.3	38 %; 17.4 ± 11.9	–
N	2 %; 2.0 ± 0.0	38 %; 2.5 ± 0.5	–
A	–	–	–
I. arboricola			
L	–	–	–
N	–	–	–
A	–	–	–
I. frontalis			
L	–	19 %; 2.0 ± 0.7	–
N	–	14 %; 1.0 ± 0.0	–
A	–	14 %; 1.0 ± 0.0	–

assumptions, standardised engorgement weight was rank-transformed. Estimates are reported as mean ± standard error unless mentioned otherwise.

Results

Of the 41 great tits, 3 (7.3 %) had ticks (average 3.00 ± 1.15 ticks per bird, range 1–5; 89 % *I. ricinus*, 11 % *I. frontalis*, no *I. arboricola*). Of the 20 blackbirds, 11 (55 %) had ticks (average 14.18 ± 9.36 ticks per bird, range 1–107; 92 % *I. ricinus*, 8 % *I. frontalis*, no *I. arboricola*). None of the 20 woodpeckers were naturally infested with ticks. Pre-experimental tick burden on the birds captured for the infestation experiment is summarised in Table 1 per tick species and life stage.

No ticks attached to woodpeckers, regardless of tick species. Attachment success (Fig. 1; Table 2) of *I. arboricola* was significantly different between host species ($\chi^2_2 = 73.94$, $p < 0.001$) but there was no effect of pre-experimental tick burden ($\chi^2_1 = 0.35$, $p = 0.557$). Post hoc testing (Table 3) indicated that attachment success of *I. arboricola* was lower on woodpeckers than on both great tits ($z = -5.74$, $p < 0.001$) and blackbirds ($z = -4.27$, $p < 0.001$). In addition, attachment success of *I. arboricola* was significantly higher on great tits than on blackbirds ($z = 4.04$, $p < 0.001$). Attachment success of *I. ricinus* was significantly different between host species ($\chi^2_2 = 42.15$, $p < 0.001$) but there was no effect of pre-experimental tick burden ($\chi^2_1 < 0.01$, $p = 0.966$). Post hoc testing indicated that attachment success of *I. ricinus* was lower on woodpeckers than on both great tits ($z = -4.27$, $p < 0.001$) and blackbirds ($z = -3.99$, $p < 0.001$), but there was no difference between great tits and blackbirds ($z = 0.27$, $p = 0.956$).

Standardised engorgement weight (Fig. 2; Table 2) of *I. arboricola* was significantly higher on great tits than on blackbirds ($\chi^2_1 = 11.34$, $p < 0.001$) but there was no effect of pre-experimental tick burden ($\chi^2_1 = 2.23$, $p = 0.135$). For *I. ricinus*, there was no significant difference between great tits and blackbirds ($\chi^2_1 = 1.51$, $p = 0.220$), nor was there an effect of pre-experimental tick burden ($\chi^2_1 = 0.27$, $p = 0.602$).

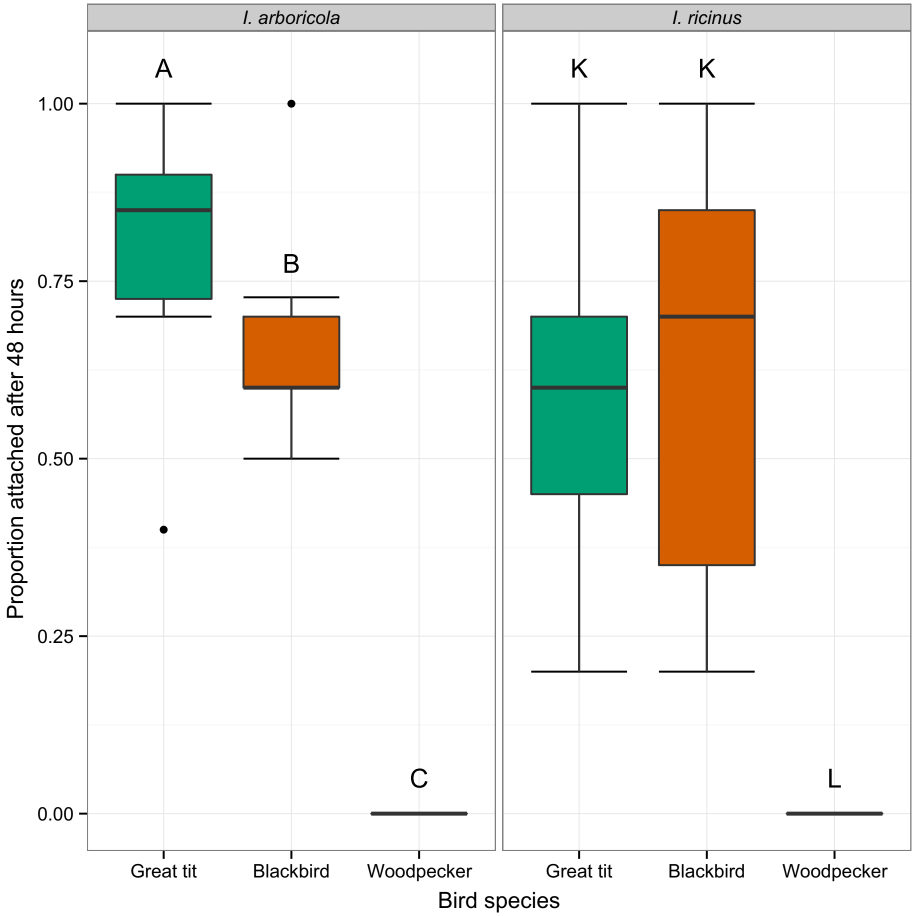

Fig. 1 Proportion of *I. arboricola* and *I. ricinus* nymphs attached to great tits, blackbirds and woodpeckers 48 h after attachment. *Letter codes* (*A*, *B*, *C*, *K*, *L*) refer to groups that do not differ significantly. *Whiskers* 1.5*IQR, *black circles* outlier values

Table 2 Summary of the fixed effects in the generalised linear mixed models of attachment success and engorgement weight in relation to bird species and pre-experimental tick burden

	Fixed effect	Attachment success		Engorgement weight	
		χ^2	*P*	χ^2	*P*
I. arboricola	Bird	$\chi^2_{2,370} = 73.94$	**<0.001**	$\chi^2_{1,120} = 11.34$	**<0.001**
	Pre-experimental burden	$\chi^2_{1,369} = 0.35$	0.557	$\chi^2_{1,119} = 2.23$	0.135
I. ricinus	Bird	$\chi^2_{2,388} = 42.15$	**<0.001**	$\chi^2_{1,152} = 1.51$	0.220
	Pre-experimental burden	$\chi^2_{1,387} < 0.01$	0.966	$\chi^2_{1,152} = 0.27$	0.602

Significant effects are given in bold

Table 3 Summary of the pairwise post hoc tests of the fixed effects in the generalised linear mixed models of attachment success of *I. arboricola* and *I. ricinus* ticks in relation to great tits, blackbirds and woodpeckers

Comparison	Attachment success		
	Estimate	*z*	*P*
IA GT × BB	−1.39 ± 0.34	−4.04	**<0.001**
IA GT × WP	−6.07 ± 1.06	−5.74	**<0.001**
IA BB × WP	−4.68 ± 1.09	−4.27	**<0.001**
IR GT × BB	0.12 ± 0.43	0.27	0.956
IR GT × WP	−5.17 ± 1.21	−4.27	**<0.001**
IR BB × WP	−5.05 ± 1.27	−3.99	**<0.001**

Significant effects are given in bold

IA Ixodes arboricola, *IR Ixodes ricinus*, *GT* great tit, *BB* blackbird, *WP* greater spotted woodpecker

Discussion

Even though some of the best-known and most widespread tick species are generalists (such as *I. ricinus* and *I. scapularis*), the realised specificity of most tick species is high, and they have a narrow host range in nature and are found on one or only a limited number of hosts (Hoogstraal and Aeschlimann 1982; Petney et al. 2011). It remains, however, unclear whether these observations of narrow host range are due to host-driven adaptations or other processes, such as habitat adaptation, behaviour and life-history characteristics (Klompen et al. 1996; for a review, see McCoy et al. 2013). We therefore conducted a study to compare infestation success among tick species with contrasting ecologies (linked to off-host microhabitat requirements) on a set of hosts that occur in both ticks' macrohabitat (forest). The endophilic specialist tick *I. arboricola* remains inside its hosts' nests and its realised host specificity is high, whereas the exophilic generalist tick *I. ricinus* actively seeks hosts and its realised host specificity is much lower. We predicted that if realised specificity is constrained by host-driven adaptations, feeding performance on novel hosts will be different from natural hosts, and therefore there are no differences between realised and potential specificity. On the other hand, if realised specificity is constrained by other processes, such as habitat adaptation and encounter filters, feeding performance will be equivalent on all hosts and hence potential specificity is lower than realised specificity.

Our experiment suggests that realised specificity of ticks is constrained by host-driven adaptations. This supports the hypothesis that potential and realised specificity are equivalent. No ticks attached to woodpeckers. As such, the fact that woodpeckers are not infested with ticks in nature is, at least partially, due to a closed compatibility filter. It remains unexplored how frequent encounters are, but the state of the encounter filter is not important when hosts are incompatible (Euzet and Combes 1980; Combes 2001). Similarly, whereas the exophilic generalist tick *I. ricinus* performed equally well on great tits and blackbirds, both of which are part of its host range, the endophilic specialist tick *I. arboricola* had lower attachment rates and weight during feeding on blackbirds, which are not part of its host range. These ticks have clearly adapted to their natural hosts. Because the number of different host species ticks encounter in nature depends on their ecology to a

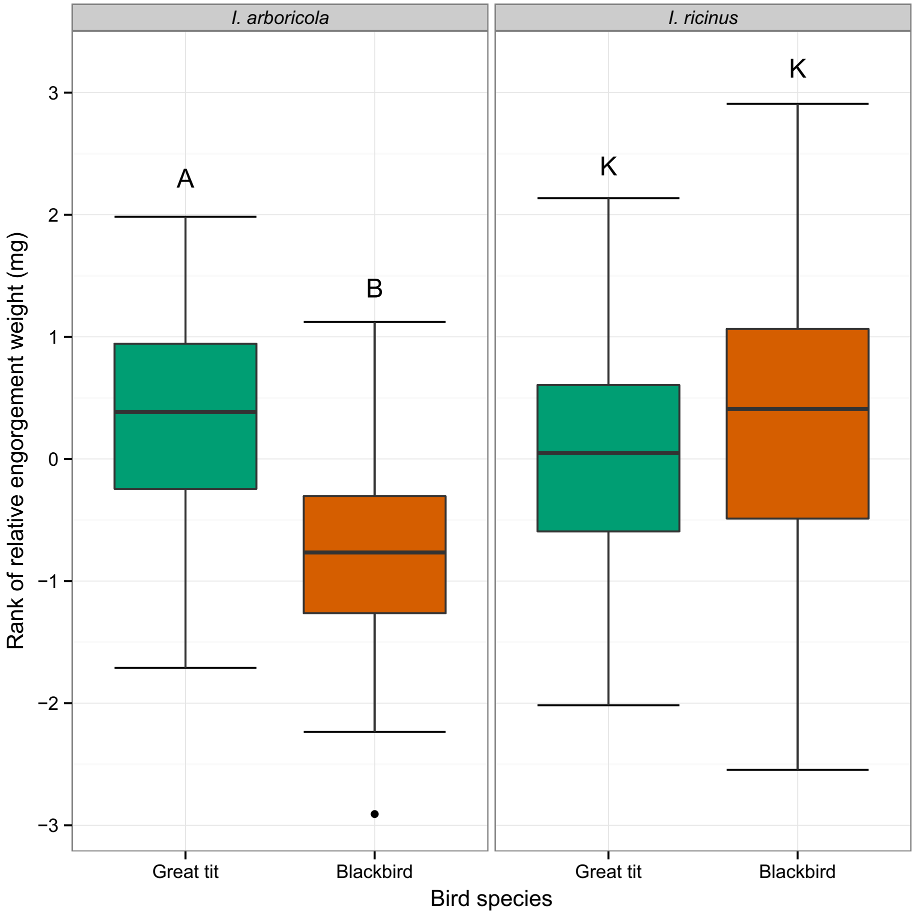

Fig. 2 Rank-transformed engorgement weight (mg), standardised across host species, of *I. arboricola* and *I. ricinus* nymphs 48 h after attachment on great tits and blackbirds. *Letter codes* (*A*, *B*, *K*) refer to groups that do not differ significantly. *Whiskers* 1.5*IQR, *black circles* outlier values

great extent, tick ecology is an important factor associated to tick-host coevolution. On the one hand, the fact that there was some attachment and engorgement of *I. arboricola* on blackbirds indicates feeding success cannot be predicted according to realised specificity.

Engorgement weight was measured by interrupting feeding after 2 days rather than after natural detachment, which occurs at least 4 days after attachment (Sonenshine 1991; Heylen and Matthysen 2010). Since we did not measure final engorgement weight, we cannot exclude the possibility that the lower blood intake might be compensated by a longer feeding duration. Nevertheless, both outcomes are likely to have a negative effect on tick fitness. Longer attachment increases tick mortality risk, e.g. due to grooming by the host as well as the risk of host mortality, whereas low engorgement weights typically lead to low moulting and hatching success and eventually egg production (Latif et al. 1988; Olegário et al. 2011). Thus, although we have not quantified survival or reproductive success of *I. arboricola* after feeding on blackbirds, the severe reduction in weight as found here gives indications that their fitness is lower on a novel host compared to a common natural host, the great tit.

Whereas ticks infest virtually all terrestrial vertebrates, the host range of the majority of tick species consists of ecologically comparable hosts (Klompen et al. 1996). Because there are often large phylogenetic differences among used host species, suggestions have been made that tick evolution has not been shaped by host-driven adaptations, but rather by ecological processes such as habitat adaptation and encounter filters (Klompen et al. 1996; McCoy et al. 2013; Nava and Guglielmone 2013). Yet, our study and a number of recent studies on potential host specificity in ticks found that ticks perform better on natural than novel hosts (Slowik and Lane 2009; Brunner et al. 2011; Harrison et al. 2012; Van Oosten et al. 2014b; Dietrich et al. 2014). It therefore seems that host specialisation has arisen over evolutionary time and ticks became specialised to the hosts used in nature, whereas the evolutionary radiation of ticks may have been determined largely by non-host factors. Off-host conditions may have played an important role in tick evolution because ticks spend the majority of their life cycle off-host (Klompen et al. 1996). There may be evolutionary feedback between tick ecology and the number of host species and types of habitat experienced by the parasite. As such, the tendency to become specialised may be much higher for endophilic than exophilic parasites. Additionally, the ecological and evolutionary consequences of an incompatible host will be much higher for endophilic parasites, which depend more on individual hosts, than exophilic parasites with high potential host availability (Hoogstraal and Aeschlimann 1982; Petney et al. 2011).

In non-tick parasite systems, it has been shown that the more different novel hosts are from the natural host, the less compatible they are. This may be in terms of geographic distribution, immunology, ecology, morphology and phylogeny (Bush and Clayton 2006; Ohhashi et al. 2007; Coile and Sikkel 2013). The little that is known about ticks infesting birds suggests that phylogeny is not a major factor for the evolution of specialisation (McCoy and Boulinier 2002; McCoy et al. 2013; Dietrich et al. 2014). Also in our study, phylogenetic relatedness seems of little importance: blackbirds are less compatible for *I. arboricola* than great tits are, whereas in a previous study we found no differences in host-tick compatibility between nestlings of great tits and the more distantly related pied flycatcher (Hackett et al. 2008; Van Oosten et al. 2014b). The implications of such a direct comparison between nestlings and adult birds may be limited because nestlings lack the resistance that adult birds potentially acquire after previous infections, although this type of resistance seems to be limited in adult birds too (Heylen et al. 2010). A second line of evidence is that we found no successful feeding on woodpeckers by *I. ricinus*, whereas the host range of this tick species includes hosts that are phylogenetically less related to songbirds than woodpeckers, e.g. a large variety of mammals and reptiles for *I. ricinus* (Matuschka et al. 1991; Gern 2005). Also for *I. arboricola*, more distantly related birds (e.g. domesticated chicken *Gallus domesticus*; Liebisch 1996) and even bats (Petney et al. 2011) serve as better hosts than woodpeckers.

The complete incompatibility of woodpeckers is also unlikely to be explained by their ecology (woodpeckers mostly excavate new cavities for roosting and breeding and mostly forage in the higher vegetation), because the encounter filter is not closed entirely for either tick species (Cramp 1985). If ecology was explanatory, we would have expected at least partial feeding success, similar to *I. arboricola* on blackbirds. Rather, great spotted woodpeckers may have specific mechanisms that make them incompatible hosts, such as an effective tick-repellent compound, immune response or grooming behaviour (Clayton et al. 2010). Even *I. ricinus*—the exemplary generalist tick of Europe—was unable to feed on woodpeckers. We claim that our study is the first to demonstrate that some terrestrial vertebrates (*in casu* woodpeckers) are incompatible hosts for *I. ricinus*.

For *I. ricinus*, the host species used in the larval stage is unknown (whereas *I. arboricola* fed on great tits under controlled conditions). Since *I. ricinus* is genetically differentiated among host species within local communities (i.e. host races; Kempf et al. 2011), there is the possibility of genetically fixed host preference and compatibility (cf. mosquitos, where experience from previous blood meals can influence the choice for subsequent hosts; Vantaux et al. 2014). To the best of our knowledge this remains untested for *I. ricinus*. If we have indeed sampled multiple host races, this may have led to variation in performance of *I. ricinus* within host species and, hence, reduced statistical power. Yet, ticks were randomised across host species and, on a species level, host races would not impair our conclusions that realised specificity of ticks is impaired by host-driven adaptations. Nevertheless, it may be worthwhile to conduct experiments regarding potential specificity with parasites reared under controlled conditions on all hosts of interest. Similarly, it could be worthwhile to use hosts with similar previous experience with ticks, a factor unknown in the current study. Yet, the effect of differential experience within bird species on our results should be limited because differential exposure should be much greater among than within host species due to contrasting ecologies. It is also important to stress that acquired immunological resistance commonly does not occur in natural ixodid tick-host interactions (Randolph 1994; Heylen et al. 2010).

In conclusion, we have shown that tick feeding performance after experimental infestation on different host species is unequal, thereby reflecting realised specificity. This suggests that the limited realised specificity generally observed in ticks may not simply be due to an encounter filter, but that compatibility filters are very important in tick evolution. Our study illustrates that we understand very little about the complex interactions between parasites and their hosts, making it difficult to predict potential specificity according to realised specificity. The integration of extensive field collection studies and experimental infestations with ectoparasites to assess realised and potential specificity, respectively, is the way forward to expand our understanding of the evolution of specialisation between hosts and ectoparasites.

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