

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

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22 Abstract

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

44 Introduction

45 The evolution of specialisation has long been of interest to evolutionary ecologists (Euzet and
46 Combes 1980; Futuyma and Moreno 1988; Kassen 2002; Poisot et al. 2011; McCoy et al.
47 2013). Specificity, which is the result of specialisation, arises because increased performance
48 in some habitats is associated with decreased performance in others due to trade-offs or
49 constraints (Kassen 2002). Similar to the concept of the niche (Hutchinson 1957), specificity
50 of an organism can be broken down into two distinct concepts. The full range of habitats
51 usable by an organism defines its potential specificity, while the observed use of these
52 habitats is its realised specificity. Potential specificity of an organism is the result of its
53 intrinsic physiological limits that allow it to establish, maintain itself and reproduce within a
54 specific range of habitats (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). In
55 contrast, realised specificity is primarily constrained by the ability to reach usable habitats
56 (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). From an evolutionary point of
57 view, potential specificity summarises the degree of adaptation to a specific range of habitats
58 and is determined by evolutionary interactions between genotype and habitat, whereas
59 realised specificity reflects the impact of ecology, chance events and history on potential
60 specificity (Bolnick et al. 2003; Devictor et al. 2010; Poisot et al. 2011). In non-parasitic
61 organisms, realised specificity is generally summarised in terms of dispersal limitation
62 (Poisot et al. 2011). In parasitic organisms, it is assumed more complex and includes spatial
63 and temporal overlap between parasites and hosts and parasite's ability to locate, invade and
64 exploit potential hosts (Poulin and Keeney 2008; McCoy et al. 2013).

65 For parasitic organisms, the degree of specificity affects both its population dynamics and its
66 evolutionary trajectory. Specificity of parasites has often been reduced to specificity towards
67 hosts (i.e. host specificity) because host specificity is arguably the most fundamental property
68 of parasitic organisms (Poulin and Keeney 2008). Traditionally, host specificity has been

69 quantified as the number of host species used under natural circumstances, and in this context
70 most parasites are considered highly host-specific (Poulin and Keeney 2008). However, such
71 high host specificity may not just reflect which host species are compatible but also which are
72 encountered, because typically fewer hosts are encountered than that could potentially be
73 compatible. Therefore the possibility to encounter a host species and host compatibility may
74 act as ‘filters’ on the evolution of host specificity (Euzet and Combes 1980; Combes 2001).
75 First, the parasite must pass through an encounter filter to reach a potential host. If successful,
76 it must also pass through a compatibility filter, determining whether the parasite can
77 circumvent or disable host defences and whether the host is nutritionally compatible for
78 parasite development. For ectoparasites, potential specificity should be determined by host
79 and off-host habitat and not just by the host as in endoparasites (Klompen et al. 1996).

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

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

106 The superfamily of ticks (Ixodoidea) encompasses approximately 800 species of
107 hematophagous ectoparasites, and realised specificity of most tick species is limited to one or
108 only a limited number of host species (Hoogstraal and Aeschlimann 1982). However, a
109 strong positive correlation was found between the degree of host specificity and sampling
110 effort, and host range might be broader than currently quantified (Klompén et al. 1996;
111 Cumming 1999; Nava and Guglielmone 2013). High potential host specificity was initially
112 expected because various structural modifications of tick mouthparts and legs are associated
113 with particular host species (Hoogstraal and Kim 1985) and because the complexity of the
114 host's immunological and behavioural responses requires host-specific circumventive
115 mechanisms (Magalhães et al. 2007). Because related species are often more similar in terms
116 of immunology, physiology and morphology, host phylogeny was attributed a considerable
117 role in the evolution of tick host specificity (Hoogstraal and Aeschlimann 1982; Hoogstraal
118 and Kim 1985). However, meta-analyses on realised specificity show weak support for

119 hypotheses that tick evolution arose through host adaptation, host specificity or cospeciation,
120 and it has been argued that realised specificity is explained much better by biogeography,
121 host ecological similarities and abiotic conditions during the long off-host period (Klompen
122 et al. 1996; Nava and Guglielmone 2013). Although a limited number of experimental studies
123 suggest that ticks generally perform better on natural than novel hosts (Slowik and Lane
124 2009; Brunner et al. 2011; Harrison et al. 2012; Dietrich et al. 2014), more investigations are
125 required to assess the importance of host-driven adaptations for realised host specificity in
126 ticks.

127 In order to evaluate the importance of potential host specificity for realised host specificity,
128 we used a laboratory experiment to investigate whether potential host specificity of two
129 sympatric tick species corresponds to their realised host specificity. *Ixodes arboricola*
130 Schulze & Schlotke 1929 is a specialist endophilic tick and its realised specificity almost
131 exclusively consists of cavity-nesting birds, predominantly great and blue tits (*Parus major*
132 L., *Cyanistes caeruleus* L.) (Walter et al. 1979; Petney et al. 2011; Heylen et al. 2014; Van
133 Oosten et al. 2014b). Endophilic ticks remain hidden inside or near to their hosts' nests or
134 burrows and only attach to the host when it arrives (Hoogstraal and Aeschlimann 1982;
135 Sonenshine 1991; Hillyard 1996). Whereas this maximises the possibility of finding a host,
136 nests are visited only by a limited variety of host species and realised host specificity of
137 endophilic ticks is high (Petney et al. 2011; Gray et al. 2014). *Ixodes ricinus* L., on the other
138 hand, is a typical generalist exophilic tick that has been recorded on a wide range of
139 vertebrate hosts, and can be found in the understorey of forests and parks (Gray 1991;
140 Hillyard 1996; Gern 2005; Marsot et al. 2012; Schulz et al. 2014). Exophilic ticks typically
141 seek hosts actively by climbing up the vegetation and waiting for them to pass (Hillyard
142 1996). Therefore exophilic ticks may encounter many different host species and, indeed,

143 realised specificity is typically lower than for endophilic ticks (Hoogstraal and Aeschlimann
144 1982; Hillyard 1996).

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

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

168 Materials & Methods

169 Study location

170 Experimental infestations took place at our laboratory and aviary at Campus Groenenborger
171 in Antwerp, Belgium in 2014. *Ixodes arboricola* came from a laboratory stock that has been
172 established in 2007 with ticks from nest boxes used by great and blue tits in woodland areas
173 near Antwerp (mainly Peerdsbos, Brasschaat). All *I. arboricola* nymphs used in the current
174 experiment fed on great tits in previous experiments. *Ixodes ricinus* were collected up to five
175 days before infestation in Grenspark de Zoom, Essen, by dragging a white flannel flag over
176 suitable vegetation. Birds were captured at Peerdsbos, Brasschaat in June and July 2014 (20
177 woodpeckers and 19 great tits) and, in September 2014, at Kalmthoutse Heide, Kalmthout (21
178 blackbirds and 2 great tits) and Campus Groenenborger, Antwerp (20 great tits). Birds
179 without a metal ring received one for identification. Birds used for previous infestations were
180 released. Each bird was inspected for ticks immediately after capture, and any attached ticks
181 were removed with tweezers for identification. All birds were housed in individual cages (8
182 m³) in an aviary at Campus Groenenborger. All cages were supplied with tree trunks as
183 climbing or perching substrates and birds had constant access to water. Woodpeckers and
184 great tits received a diet of mealworms, fat balls, peanuts and sunflower seeds *ad libitum*.
185 Blackbirds received a diet of mealworms, insect pate, dried bread and apples *ad libitum*.
186 Cages for woodpeckers and great tits were provided with a nest box (3.4 dm³ for great tits,
187 42.9 dm³ for woodpeckers).

188

189 Ticks – *Ixodes arboricola*, *Ixodes ricinus*

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

200 *Ixodes ricinus* is an exophilic generalist tick found in deciduous woodland, meadows and
201 moorland across Europe, Russia, North Africa and the Middle East (Gern 2005; Petney et al.
202 2011). It climbs up to some vantage point in the lower vegetation (“questing”) from where it
203 contacts passing vertebrate hosts. To ensure detachment in the field rather than a cavity when
204 infesting hosts that make use of cavities, it detaches predominantly during the day (Heylen
205 and Matthysen 2010 and references therein). Immature life stages infest a wide array of
206 terrestrial vertebrates, including songbirds, although adults are only found on larger animals
207 (Gray 1991; Gern 2005).

208

209 Avian hosts – great tit, blackbird, great spotted woodpecker

210 The great tit (family Paridae), the commonest secondary cavity-nesting bird in Western
211 Europe, uses yet available natural or artificial cavities for breeding and roosting (Gosler
212 1993) and is therefore frequently exposed to *I. arboricola* (Literák et al. 2007; Heylen et al.

213 2014). The foraging niche includes the lower vegetation strata inside forests, resulting in
214 frequent *I. ricinus* infestations, especially during the breeding season (Heylen et al. 2014).

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

221 The great spotted woodpecker (family Picidae), a widespread breeding bird in Europe mostly
222 found in mature forests, usually excavates new cavities for breeding and roosting (Cramp
223 1985). Generally woodpeckers do not forage on the ground but they may do this in fall and
224 winter to collect seeds and nuts (Cramp 1985). So far, no ticks have been reported on great
225 spotted woodpeckers (Literák et al. 2007; Marsot et al. 2012; Norte et al. 2012). In our own
226 capture sessions in Peerdsbos, Brasschaat, 101 great spotted woodpeckers were inspected for
227 ticks over a five-year period, but ticks were never found, whereas great tits were frequently
228 infested with either tick species (unpublished results).

229

230 Experimental infestations

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

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

245

246 Statistical analyses

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

257 Fixed effects in all models included: bird species (blackbird and great tit; woodpecker in the
258 model for attachment success) and the number of ticks found on birds pre-experimentally
259 (log-transformed; Table 1). In all models a stepwise selection procedure was used in which

260 the model was iteratively refitted after exclusion of the least significant fixed effect. Post hoc
261 tests were conducted the main effect bird species with package multcomp v 1.4-0, which
262 corrects for multiple comparisons with Tukey Contrasts (Hothorn et al. 2008). Because
263 attachment success across all woodpeckers was zero, variance estimates could not be
264 calculated and the models did not converge. This was resolved by using a dummy variable, in
265 which attachment of a single nymph of both tick species was changed to “success”, hence
266 introducing artificial variance. This had no effect on the results due to the large sample size
267 (*I. ricinus* $N = 100$; *I. arboricola* $N = 90$). To fulfil normality assumptions, standardised
268 engorgement weight was rank-transformed. Estimates are reported as mean \pm standard error
269 unless mentioned otherwise.

270 Results

271 Of the 41 great tits, 3 (7.3%) had ticks (average 3.00 ± 1.15 ticks per bird, range 1 – 5; 89% *I.*
272 *ricinus*, 11% *I. frontalis*, no *I. arboricola*). Of the 20 blackbirds, 11 (55%) had ticks (average
273 14.18 ± 9.36 ticks per bird, range 1 – 107; 92% *I. ricinus*, 8% *I. frontalis*, no *I. arboricola*).
274 None of the 20 woodpeckers were naturally infested with ticks. Pre-experimental tick burden
275 on the birds captured for the infestation experiment is summarised in Table 1 per tick species
276 and life stage.

277 No ticks attached to woodpeckers, regardless of tick species. Attachment success (Fig. 1,
278 Table 2) of *I. arboricola* was significantly different between host species ($\chi^2_2 = 73.94$, $p <$
279 0.001) but there was no effect of pre-experimental tick burden ($\chi^2_1 = 0.35$, $p = 0.557$). Post
280 hoc testing (Table 2) indicated that attachment success of *I. arboricola* was lower on
281 woodpeckers than on both great tits ($z = -5.74$, $p < 0.001$) and blackbirds ($z = -4.27$, $p <$
282 0.001). In addition, attachment success of *I. arboricola* was significantly higher on great tits
283 than on blackbirds ($z = 4.04$, $p < 0.001$). Attachment success of *I. ricinus* was significantly
284 different between host species ($\chi^2_2 = 42.15$, $p < 0.001$) but there was no effect of pre-
285 experimental tick burden ($\chi^2_1 < 0.01$, $p = 0.966$). Post hoc testing indicated that attachment
286 success of *I. ricinus* was lower on woodpeckers than on both great tits ($z = -4.27$, $p < 0.001$)
287 and blackbirds ($z = -3.99$, $p < 0.001$), but there was no difference between great tits and
288 blackbirds ($z = 0.27$, $p = 0.956$).

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

294 Discussion

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

312 Our experiment suggests that realised specificity of ticks is constrained by host-driven
313 adaptations. This supports the hypothesis that potential and realised specificity are equivalent.
314 No ticks attached to woodpeckers. As such, the fact that woodpeckers are not infested with
315 ticks in nature is, at least partially, due to a closed compatibility filter. It remains unexplored
316 how frequent encounters are, but the state of the encounter filter is not important when hosts
317 are incompatible (Euzet and Combes 1980; Combes 2001). Similarly, whereas the exophilic
318 generalist tick *I. ricinus* performed equally well on great tits and blackbirds, both of which

319 are part of its host range, the endophilic specialist tick *I. arboricola* had lower attachment
320 rates and weight during feeding on blackbirds, which are not part of its host range. These
321 ticks have clearly adapted to their natural hosts. Because the number of different host species
322 ticks encounter in nature depends on their ecology to a great extent, tick ecology is an
323 important factor associated to tick-host coevolution. On the one hand, the fact that there was
324 some attachment and engorgement of *I. arboricola* on blackbirds indicates feeding success
325 cannot be predicted according to realised specificity.

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

337 Whereas ticks infest virtually all terrestrial vertebrates, the host range of the majority of tick
338 species consists of ecologically comparable hosts (Klompen et al. 1996). Because there are
339 often large phylogenetic differences among used host species, suggestions have been made
340 that tick evolution has not been shaped by host-driven adaptations, but rather by ecological
341 processes such as habitat adaptation and encounter filters (Klompen et al. 1996; McCoy et al.
342 2013; Nava and Guglielmone 2013). Yet, our study and a number of recent studies on
343 potential host specificity in ticks found that ticks perform better on natural than novel hosts

344 (Slowik and Lane 2009; Brunner et al. 2011; Harrison et al. 2012; Van Oosten et al. 2014b;
345 Dietrich et al. 2014). It therefore seems that host specialisation has arisen over evolutionary
346 time and ticks became specialised to the hosts used in nature, whereas the evolutionary
347 radiation of ticks may have been determined largely by non-host factors. Off-host conditions
348 may have played an important role in tick evolution because ticks spend the majority of their
349 life cycle off-host (Klompen et al. 1996). There may be evolutionary feedback between tick
350 ecology and the number of host species and types of habitat experienced by the parasite. As
351 such, the tendency to become specialised may be much higher for endophilic than exophilic
352 parasites. Additionally, the ecological and evolutionary consequences of an incompatible host
353 will be much higher for endophilic parasites, which depend more on individual hosts, than
354 exophilic parasites with high potential host availability (Hoogstraal and Aeschlimann 1982;
355 Petney et al. 2011).

356 In non-tick parasite systems, it has been shown that the more different novel hosts are from
357 the natural host, the less compatible they are. This may be in terms of geographic distribution,
358 immunology, ecology, morphology and phylogeny (Bush and Clayton 2006; Ohhashi et al.
359 2007; Coile and Sikkell 2013). The little that is known about ticks infesting birds suggests that
360 phylogeny is not a major factor for the evolution of specialisation (McCoy and Boulinier
361 2002; McCoy et al. 2013; Dietrich et al. 2014). Also in our study, phylogenetic relatedness
362 seems of little importance: blackbirds are less compatible for *I. arboricola* than great tits are,
363 whereas in a previous study we found no differences in host-tick compatibility between
364 nestlings of great tits and the more distantly related pied flycatcher (Hackett et al. 2008; Van
365 Oosten et al. 2014b). The implications of such a direct comparison between nestlings and
366 adult birds may be limited because nestlings lack the resistance that adult birds potentially
367 acquire after previous infections, although this type of resistance seems to be limited in adult
368 birds too (Heylen et al. 2010). A second line of evidence is that we found no successful

369 feeding on woodpeckers by *I. ricinus*, whereas the host range of this tick species includes
370 hosts that are phylogenetically less related to songbirds than woodpeckers, e.g. a large variety
371 of mammals and reptiles for *Ixodes ricinus* (Matuschka et al. 1991; Gern 2005). Also for *I.*
372 *arboricola*, more distantly related birds (e.g. domesticated chicken *Gallus domesticus*;
373 Liebisch 1996) and even bats (Petney et al. 2011) serve as better hosts than woodpeckers.
374 The complete incompatibility of woodpeckers is also unlikely to be explained by their
375 ecology (woodpeckers mostly excavate new cavities for roosting and breeding and mostly
376 forage in the higher vegetation), because the encounter filter is not closed entirely for either
377 tick species (Cramp 1985). If ecology was explanatory, we would have expected at least
378 partial feeding success, similar to *I. arboricola* on blackbirds. Rather, great spotted
379 woodpeckers may have specific mechanisms that make them incompatible hosts, such as an
380 effective tick-repellent compound, immune response or grooming behaviour (Clayton et al.
381 2010). Even *I. ricinus* - the exemplary generalist tick of Europe - was unable to feed on
382 woodpeckers. We claim that our study is the first to demonstrate that some terrestrial
383 vertebrates (*in casu* woodpeckers) are incompatible hosts for *I. ricinus*.

384 For *I. ricinus*, the host species used in the larval stage is unknown (whereas *I. arboricola* fed
385 on great tits under controlled conditions). Since *I. ricinus* is genetically differentiated among
386 host species within local communities (i.e. host races; Kempf et al. 2011), there is the
387 possibility of genetically fixed host preference and compatibility (cf. mosquitos, where
388 experience from previous blood meals can influence the choice for subsequent hosts; Vantaux
389 et al. 2014). To the best of our knowledge this remains untested for *I. ricinus*. If we have
390 indeed sampled multiple host races, this may have led to variation in performance of *I.*
391 *ricinus* within host species and, hence, reduced statistical power. Yet, ticks were randomised
392 across host species and, on a species level, host races would not impair our conclusions that
393 realised specificity of ticks is impaired by host-driven adaptations. Nevertheless, it may be

394 worthwhile to conduct experiments regarding potential specificity with parasites reared under
395 controlled conditions on all hosts of interest. Similarly, it could be worthwhile to use hosts
396 with similar previous experience with ticks, a factor unknown in the current study. Yet, the
397 effect of differential experience within bird species on our results should be limited because
398 differential exposure should be much greater among than within host species due to
399 contrasting ecologies. It is also important to stress that acquired immunological resistance
400 commonly does not occur in natural ixodid tick-host interactions (Randolph 1994; Heylen et
401 al. 2010).

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

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575

576 Legends to Figures

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

580

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

585

586 **S1** Histogram of pre-experimental tick burden on great tits, blackbirds and woodpeckers.
587 Data are pooled for tick species and instars. One blackbird infested with 104 ticks is not
588 shown

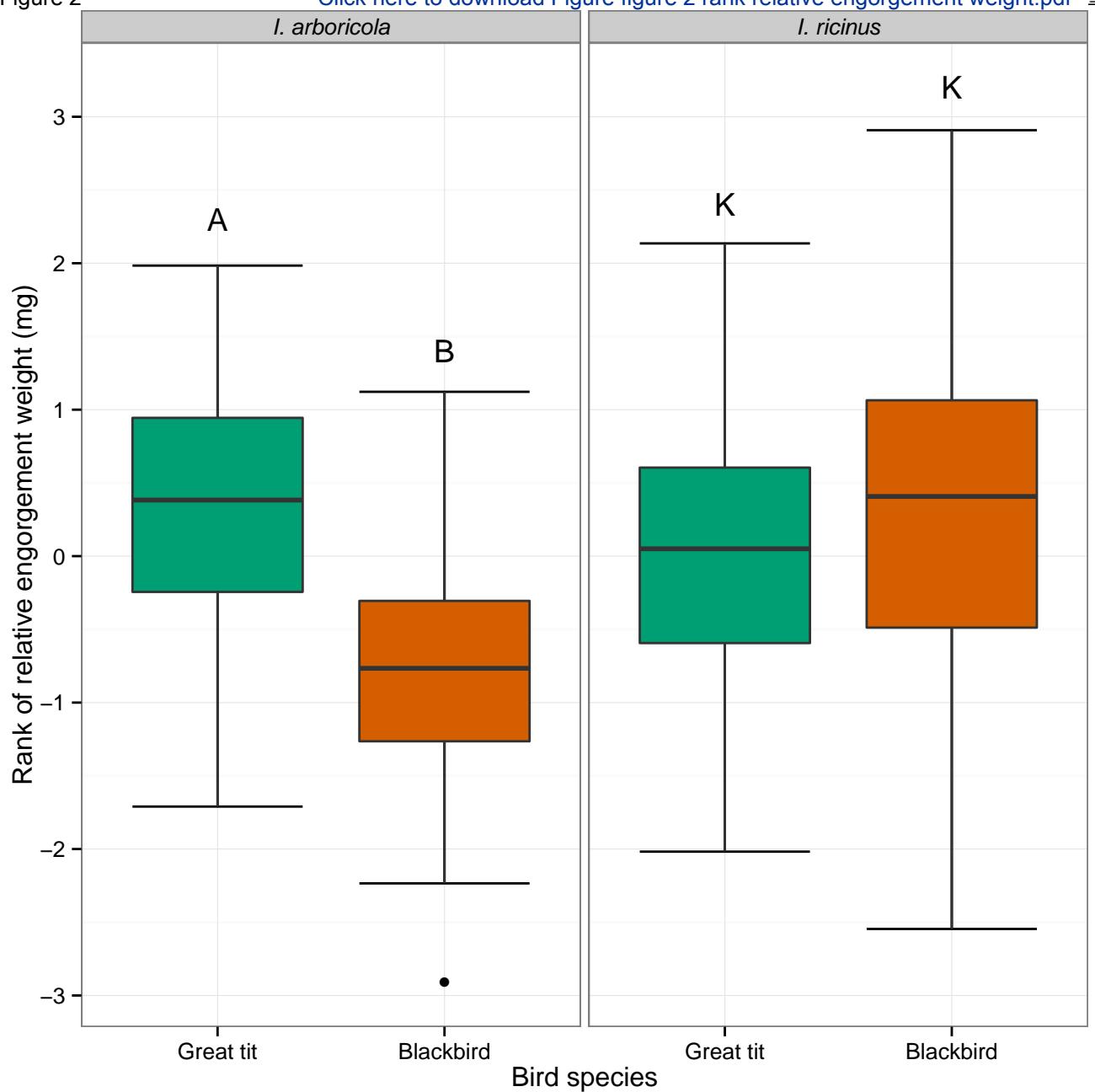


Table 1 Pre-experimental infestation intensity and number of ticks \pm SE on infested birds per instar (larva, nymph, adult) of *I. ricinus*, *I. arboricola* and *I. frontalis* on great tits, blackbirds and woodpeckers.

		Great tit	Blackbird	Woodpecker
<i>I. ricinus</i>	L	7%; 2.3 \pm 1.3	38%; 17.4 \pm 11.9	-
	N	2%; 2.0 \pm 0.0	38%; 2.5 \pm 0.5	-
	A	-	-	-
<i>I. arboricola</i>	L	-	-	-
	N	-	-	-
	A	-	-	-
<i>I. frontalis</i>	L	-	19%; 2.0 \pm 0.7	-
	N	-	14%; 1.0 \pm 0.0	-
	A	-	14%; 1.0 \pm 0.0	-

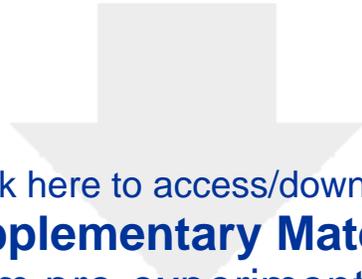
Table 1 Summary of the fixed effects in the generalised linear mixed models of attachment success and engorgement weight in relation to bird species and pre-experimental tick burden. Significant effects are given in bold.

Fixed effect		attachment success		engorgement weight	
		χ^2	<i>P</i>	χ^2	<i>P</i>
<i>I. arboricola</i>	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>I. ricinus</i>	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

Table 2 Summary of the pairwise post hoc tests of the fixed effects in the generalised linear mixed models of attachment success of *Ixodes arboricola* and *I. ricinus* ticks in relation to great tits, blackbirds and woodpeckers. Significant effects are given in bold.

comparison	attachment success		
	<i>estimate</i>	<i>z</i>	<i>P</i>
IA GT x BB	-1.39 ± 0.34	-4.04	< 0.001
IA GT x WP	-6.07 ± 1.06	-5.74	< 0.001
IA BB x WP	-4.68 ± 1.09	-4.27	< 0.001
IR GT x BB	0.12 ± 0.43	0.27	0.956
IR GT x WP	-5.17 ± 1.21	-4.27	< 0.001
IR BB x WP	-5.05 ± 1.27	-3.99	< 0.001

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



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Supplementary Material

supp histogram pre-experimental burdens.pdf

