

Research

Heritable variation in host quality as measured through an ectoparasite's performance

Gerardo Fracasso, Erik Matthysen and Dieter Heylen

G. Fracasso (<https://orcid.org/0000-0003-3384-4472>) ✉ (gerardo.fracasso@uantwerpen.be) and E. Matthysen (<https://orcid.org/0000-0002-7521-9248>), Dept of Biology, Univ. of Antwerp, Wilrijk, Belgium. – D. Heylen (<https://orcid.org/0000-0002-6136-8007>), Interuniversity Inst. for Biostatistics and Statistical Bioinformatics, Hasselt Univ., Diepenbeek, Belgium and Eco-Epidemiology Group, Dept of Biomedical Sciences, Inst. of Tropical Medicine, Antwerp, Belgium.

Oikos

00: 1–14, 2021

doi: 10.1111/oik.08824

Subject Editor: Sara Magalhães

Editor-in-Chief:

Gerlinde B. De Deyn

Accepted 14 November 2021



Obligate parasites need one or more hosts to complete their life cycle. However, hosts might show intraspecific variation in quality with respect to the parasites themselves, thus affecting on-host and off-host parasite performance. High heritability in host quality for the parasite may therefore exert long-lasting selective pressures on the parasite and influence host–parasite coevolution. However, the amount of variation and heritability in host quality are unknown for most parasite species, especially in wild populations of hosts. Both measures were estimated in a wild-caught bird *Parus major* that was experimentally infested by two developmental stages (larva and nymph) of a ectoparasite (the tick *Ixodes arboricola*). We examined variation in host quality through variation in tick performance, namely the on-host performance (attachment success, feeding time, engorgement weight and feeding success) and the off-host performance (moulting time, moulting success and overall survival). Herein we also investigated the influence on tick performance of host traits linked with the bird's life history and physiology such as body condition, sex, age and haematocrit. By correlating tick performance variables between larvae and nymphs feeding on the same bird at different times, we found a significant correlation in attachment success, suggesting consistent among-host variation for this performance measure, but no significant larva–nymph correlations for the other tick variables. Animal models relating tick performance variables to the host pedigree showed a strong heritable signal for host quality as measured through tick feeding time, and lower but substantial estimates in other performance variables. With regard to the host traits, feeding success and survival of tick larvae were lower on female birds, and nymphal survival was higher on older birds. Larval feeding time was negatively correlated with host haematocrit. This is one of the first studies showing consistent intraspecific variation and heritability of host quality for a multi-stage ectoparasite.

Keywords: host quality, host–parasite coevolution, *Ixodes arboricola*, life-history traits, *Parus major*, trait heritability



www.oikosjournal.org

© 2021 The Authors. Oikos published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Introduction

Parasites need to feed on a host to successfully complete their life cycle and hosts counteract such exploitation with behavioural (Bush and Clayton 2018, Hart and Hart 2018, Sarabian et al. 2018), morphological (Clayton et al. 2005, Villa et al. 2018) and immune defences (Jo 2019). From the perspective of the parasite, hosts might vary in the strength of host defence and nutritional value at both inter- and intraspecific level, and thus differentially affect parasite performance and ultimately parasite fitness (Christe et al. 2003, Bize et al. 2008, Heylen and Matthysen 2011a). A number of traits within a host species have been shown to affect parasite performance, such as host body mass and condition (Cornet et al. 2014), age (Christe et al. 2007, Lourenço and Palmeirim 2008, Izhar and Ben-Ami 2015), sex (Sanchez et al. 2011, Roberts and Hughes 2015) and haematocrit (i.e. the proportion of blood consisting of red blood cells; Taylor and Hurd 2001); and often parasite performance relates to them in a complex way (Tschirren et al. 2007, Jones et al. 2015). Here we investigate variation in host quality from the parasite perspective, where we define quality as the characteristics of the host that increase parasite performance. The mechanisms underlying variation in host quality are related to the non-mutually exclusive concepts of resistance and tolerance. Host resistance is the ability to reduce parasite burden and can be achieved through behavioural, morphological and immune adaptations that reduce parasite fitness. Tolerance is instead the ability to reduce the harm caused by the parasite, often by means of physiological adaptations, without necessarily impacting parasite fitness (Råberg et al. 2009).

Individual variation in host quality from the parasite's perspective and its underlying drivers have rarely been studied. Moreover, despite broad evidence for host–parasite coevolution (Gagneux 2012, Masri et al. 2013, Clayton et al. 2015, Paplauskas et al. 2021), very few studies have examined heritable variation in host quality, i.e. the degree to which variation in parasite performance is explained by host genetic background, especially in wildlife hosts and their parasites (Smith et al. 1999, Mazé-Guilmo et al. 2014). Nevertheless, although the causal mechanistic physiological relationships between host traits and parasite fitness are not completely understood, the investigation of host trait variation and its linkage with parasite performance are an essential (Barrett et al. 2008) – but very often overlooked – first step in the exploration of mutual selection pressures underpinning host–parasite interactions and coevolution (Best et al. 2009, Carval and Ferriere 2010). In fact, substantial repeatability and heritability in host quality are requirements for the evolution of host defence in response to parasite pressure. Moreover, improving our understanding of how host quality affects parasite performance may also contribute to the effectiveness of anti-parasite management (Nauen 2007, Hemingway et al. 2016, Yessinou et al. 2016).

Ectoparasites feeding on the host surface offer a remarkable study system to investigate host–parasite interactions and (co)evolution, as they exert selective pressures on their

hosts and their traits can easily be measured (Clayton and Moore 1997, Poulin 2007). In some parasite groups individuals are sufficiently large to allow monitoring of life-history and other performance variables (on- and off-host) at the individual level (Dlugosz et al. 2014, Bush et al. 2019). The study of individual host variation can therefore be potentially performed in every developmental stage of those ectoparasites, enabling the investigation of the trade-offs and selective pressures associated with the reciprocal evolutionary changes between host and parasite (Clutton-Brock and Sheldon 2010, Clayton et al. 2015). In the wild, most ectoparasite species are unevenly distributed on hosts, with few hosts contributing to most of the parasite population (Clayton and Moore 1997, Poulin 2007, Clayton et al. 2015). This pattern also suggests differences in host quality but a number of extrinsic confounding factors (e.g. unequal parasite exposure) cannot be ruled out in wild conditions.

In this study we investigate individual variation and heritability in host quality with respect to ectoparasite performance in a well-studied songbird–parasite system, the great tit *Parus major* and the tree-hole tick *Ixodes arboricola* (Heylen et al. 2014b, Van Oosten et al. 2014b, 2016, 2018). Even though *Ixodes arboricola* has negligible effects on host health (below) we nevertheless consider it a parasite since it feeds, by taking a bloodmeal, at the expenses of its host and therefore must inflict some (minimal) harm (Combes 2001, Poulin 2007). This unique system permits the tracking of both the host and parasite at the individual level. Similarly to most ectoparasites, tree-hole ticks show an aggregated distribution in their host populations, including in our study area (Supporting information; Heylen et al. 2014b). To investigate intrinsic differences in host quality – excluding extrinsic confounding factors – we carried out standardized infestations in the lab. For each bird individual, we quantified host quality through the performance of the ticks feeding on it. Importantly, *I. arboricola* has negligible impact on the health of great tit hosts (Heylen and Matthysen 2011b, Van Oosten et al. 2016). Hence, host traits measured during an experimental infestation (such as body weight and haematocrit) can be considered as largely unaffected by the tick itself. Furthermore, great tits do not show acquired immunological resistance against congeneric *Ixodes ricinus* ticks (Heylen et al. 2010, 2021) – as is often the case for hosts exposed to ticks with whom they have a coevolutionary history (Karasuyama et al. 2020). We measured a suite of variables related to parasite performance that we split up into on-host parasite performance on the one hand, i.e. variables related to the host exploitation, and off-host parasite performance in the other hand, i.e. variables related to parasite development and survival to the next stage (an overview is presented in Fig. 1). Since many birds were typically infested once by larvae, and on another occasion by nymphs, individual variation in host quality was assessed as the within-host across-stage correlation in tick performance. Heritability of host quality was evaluated by linking tick performance to the genetic relatedness between individual birds derived from a pedigree containing all ringed birds in the population. Finally, we explored whether variation in parasite

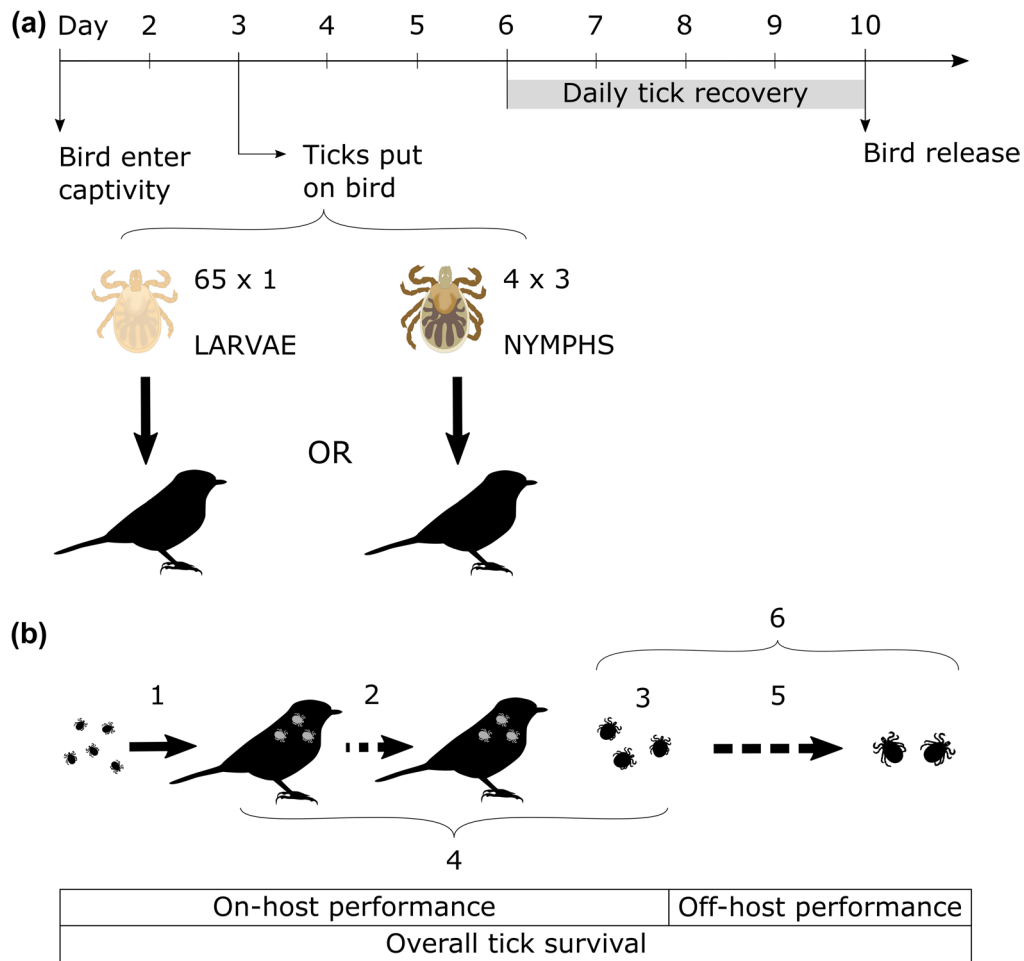


Figure 1. Study design with definition of tick variables. (a) Overview of the experimental infestations on adult great tits. After two days of acclimatization every bird was infested with either 65 larvae from one clutch or 12 nymphs from 3 clutches (4 ticks from each clutch). (b) Overview of tick performance measures. On-host variables: (1) attachment success, (2) feeding time, (3) engorgement weight, (4) feeding success; and off-host variables: (5) moulting time, (6) moulting success.

performance among hosts could be explained by host sex, age, body condition (and its change over the captivity period) and haematocrit.

Material and methods

Study species

Between 2017 and 2020 two consecutive generations of the nidicolous tree-hole tick *Ixodes arboricola* were reared in laboratory conditions and fed on wild great tits *Parus major*. Larvae and nymphs were allowed to engorge on adult birds temporarily brought into captivity while adult females (not analysed in this study) engorged on nestlings in the wild (Fracasso et al. 2022). Tree-hole ticks feed once per life stage (except adult males) and are specialized on cavity-nesting birds, in particular great and blue tits *Cyanistes caeruleus* (White et al. 2012, Sonenshine and Roe 2013, Heylen et al. 2014b, Van Oosten et al. 2014a). Immature *I. arboricola*

stages naturally feed throughout the year whenever birds use cavities (Heylen et al. 2014b).

Great tits are small songbirds preferentially breeding in deciduous woodlands and widespread across Europe, part of Asia and North Africa (Cramp and Perrins 1993). The birds used in this study were part of a wild population settled in the Boshoeke area (51°7'59"N, 4°31'1"E) near Antwerp (Belgium) and breeding in nest boxes (see Matthysen 2002 for details on the study site). This population is part of a long-term study and as such most of the resident birds are of known age and their genetic relatedness is known. Specifically, every year all parents breeding in nest boxes are identified by capturing them at the nest and nestlings are individually ringed before they fledge. Consequently, more than 50% of breeders have known parents, i.e. previously bred in the same area (Korsten et al. 2013). A combination of empirical data and simulation studies shows that heritability estimates from field-based pedigrees are relatively robust to misassignments due to extra-pair paternity (Firth et al. 2015).

Study design

In 2017, 54 adult *I. arboricola* females and 58 adult males were collected from four wooded areas (including the above-mentioned one) within a 25 km distance from the centre of Antwerp to establish a lab population (Van Oosten et al. 2014b). Two consecutive complete tick generations were raised in semi-natural conditions and individually followed throughout their three life stages (see also Fracasso et al. 2022). Ticks were kept in darkness at 20°C and 85% relative humidity when not feeding on the birds.

In October–December (larvae) and January–February (nymphs) ticks were fed on full-grown great tits temporarily held in cages equipped with a standard nest box for 10 days. Cohorts of larvae or nymphs were usually split over multiple 10-day long infestation periods involving a maximum of 24 different birds, each of them henceforth called ‘batch’ (Fig. 1). Each batch was given a unique number. Birds were caught from the wild prior to every infestation batch and immediately released afterwards. Ticks put on a specific bird within a specific batch will be referred to as being part of a single ‘feeding event’. Before infestation every bird was given at least 48 h to acclimatise. During our study, wild-caught great tits were occasionally infested with wild *I. ricinus* and *I. arboricola* ticks, mostly at low infestation intensities. Hence, birds were briefly inspected just prior to the experimental infestation and any wild tick was removed. The time between catching and experimental infestations (at least 48 h) allowed to most of these wild ticks to detach prior to the experimental infestation or to be easily spotted at inspection due to their stage of engorgement. Ticks were put on the head of birds using a paintbrush (larvae) or tweezers (nymphs) in accordance to the natural attachment behaviour of ixodid ticks (Fracasso et al. 2019) and earlier studies (Heylen and Matthysen 2010, Heylen et al. 2014a, 2017). Each bird received approximately 65 larvae from the same clutch, or exactly 12 nymphs evenly representing 3 different clutches (4 nymphs for every clutch). Immediately afterwards, birds were put singly in an air-permeable cotton bag for one hour to optimize tick attachment (Heylen et al. 2017, Fracasso et al. 2019). Nymphs were individually marked by clipping part of one limb (except the first pair holding the Haller’s organ) with a scalpel within 2 h prior to infestation. Tick identity was verified immediately after engorgement. Tree-hole ticks show a striking tendency to detach inside cavities or nest boxes (White et al. 2012). To collect them, nest box inspection was performed daily for five consecutive days starting from the third day after infestation, corresponding to natural *I. arboricola* detachment time. Ticks found still attached to the bird one week after infestation, i.e. just prior to bird release, were considered as having fed for one additional day.

Parasite performance variables

Tick performance variables were divided in two main groups: on-host and off-host tick performance. On-host parasite

performance variables were: attachment success, feeding time, engorgement weight and feeding success. Off-host parasite variables were: moulting time and moulting success. We also included overall parasite survival from initial infestation until (and including) moulting. We assume that a higher host quality is associated with higher success rates and higher engorgement weight. We also expect that longer moulting times reflect a more difficult conversion of the blood meal and hence lower host quality. Similarly, hosts of low quality are expected to slow down tick feeding thus leading to longer feeding times. As regards tick success ratios, attachment success was defined as the proportion of ticks not found in the bag after one hour from infestation, hence presumably attached, relative to all ticks put on the bird. In this way, we also accounted for ticks that attached but did not complete engorgement and were therefore missed later on. We specified feeding success as the proportion of ticks presumably attached that were recovered engorged. Moulting success was defined as the proportion of moulted ticks with respect to the number of engorged ticks recovered from each bird. We also measured overall tick survival, namely the combined outcome of on- and off-host survival, as the proportion of ticks that moulted into the next life stage relative to all ticks put on the bird. Hence, survival combines all previous success ratios: attachment, feeding and moulting success.

Tick feeding time was calculated as the number of days between infestation and collection. Engorgement weight was measured twice to the nearest 10^{-2} mg and the average value was then used in the analyses. We defined moulting time as the number of days elapsed between tick detachment and emergence from the exuvia (ecdysis). We defined fasting time as the number of days between the experimental infestation and either hatching from the egg (larvae) or detachment as larva from the previous feeding event (nymphs) and included this as a covariate, since this time period was set by the experimenter and not by the tick. Longer fasting times imply fewer resources available to successfully attach and initiate feeding. Feeding density, namely the number of ticks presumed to be attached, was also included as a covariate.

Host traits

As ixodid ticks feed during a non-stop period of several days, we chose to focus on host traits that could be recorded without interfering with the tick’s feeding process, which is also why we did not take blood samples prior to infestation. Birds were weighed three times: 1) at capture, 2) on the fourth day after infestation (i.e. at the peak of tick detachment), 3) at release. Body condition was expressed relative to tarsus length using the scaled mass index, for males and females separately (Peig and Green 2009, 2010). To calculate the scaling exponent we used all capture data (both roosting and mist netting) from the bird population used in this study since 1997 (11 468 males and 10 645 females). Previous studies have shown that bird body condition is related to survival (Naef-Daenzer et al. 2001, Krams et al. 2010), immune response (Navarro et al. 2003, Bowers et al. 2014) and parasite feeding

success (Dube et al. 2018). Since our second measure of body condition (on the fourth day after infestation) and the third one (at release) were highly correlated ($R=0.89$, $p < 0.001$), the latter was not used in further analyses. Host age (in years) was measured by hatching date while sex was assessed by plumage characteristics (Cramp and Perrins 1993).

Before bird release, a blood sample was taken using a heparinized capillary (60 μ l) and all ticks still attached were removed. To safeguard bird health we decided beforehand that birds with a body weight lower than 15 g were excluded from blood sampling. For this reason, haematocrit was not taken in 103 feeding events out of 255. Capillaries were then centrifuged for 10 min at 14 000 g. Haematocrit level was measured as the length of the capillary occupied by red blood cells over the total length of blood in the capillary by using a digital caliper to the nearest 0.01 mm (Heylen and Matthysen 2011b). Haematocrit is a measure of the oxygen-carrying capacity (Minias 2020) and viscosity of blood (Birchard 1997).

Statistical analysis

Since ticks were marked individually only in the nymph stage, we defined all performance measures at the level of the feeding event, i.e. the mean value of each performance variable for all ticks on a single bird in the same infestation. Data were analysed in R ver. 4.0.5 (<www.r-project.org>). To check model assumptions we plotted the distribution of the standardized deviance residuals and checked for the presence of outliers using the 'DHARMa' (ver. 0.3.3.0) package (Hartig 2020). All models described below are generalized linear mixed models (or a subgroup of them) and are described by the following equation:

$$\mathbf{y} = \mathbf{X} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$$

where \mathbf{y} is the response variable as a $N \times 1$ vector (in our study a tick performance variable); \mathbf{X} is a $N \times p$ matrix of the p fixed effects (predictor variables) with $\boldsymbol{\beta}$ being a fixed-effects vector of the regression coefficients; \mathbf{Z} is a $N \times q$ matrix of the q random effects with \mathbf{u} being a random-effects vector and $\boldsymbol{\varepsilon}$ a vector of the residuals, i.e. the part of variation of \mathbf{y} not explained by the model.

Between-stage correlations

We investigated the between-stage correlations for every tick on-host (attachment success, feeding time, engorgement weight and feeding success) and off-host (moulting time, moulting success) performance variable as well as overall survival, on individual hosts. If individual birds vary in host quality, we expect tick performance to be correlated between larval and nymph infestations on the same bird. In total, 25 birds were infested once with larvae and once with nymphs. Three of these birds were infested three times: twice with the same life stage and once with the other stage. All repeated infestations were carried out in different periods (batches). A

few birds were repeatedly infested with the same stage: three with larvae and six with nymphs. This was because larva and nymph infestations were done at different times of the year and we avoided to repeatedly infest the same bird within a month. Hence, we could not investigate within-stage correlations due to the low sample size. The time interval between the first and second infestation, once with larvae and once with nymphs, ranged between 49 and 315 days. To calculate the within-host correlation in larval and nymph performance, we fitted a Bayesian linear mixed model for each tick performance variable in the 'brms' (ver. 2.15.0) package (Bürkner 2017, 2018). We ran four chains in parallel with default weakly informative priors. Larval and nymph performance variables were treated as separate response variables in the same model (bivariate models). For all performance variables we set a normal error distribution function. This allowed us to use the correlation between the residuals as a measure of within-host correlation between larvae and nymphs for a given tick performance variable. Assumptions of normality were tested using the Shapiro–Wilk test and variables violating the assumptions were normalized using the best transformation according to the 'bestNormalize' (ver. 1.7.0) package (Peterson and Cavanaugh 2019). Batch was set as random effect, thereby accounting for any temporal variation between and within years. We did not include any fixed effect in order to investigate the correlation between stages irrespective of other confounding factors. Model convergence and autocorrelation were checked following the guidelines of Wilson et al. (2010) and de Villemereuil (2018) by means of the diagnostic functions embedded in the 'brms' package to analyse the posterior distributions, chain autocorrelations and efficiency of the sampler.

Heritability

To investigate the effect of host genetic background on tick performance we fitted a Bayesian animal model for each tick performance variable and life stage. Animal models make use of a matrix of genetic relatedness between individuals (pedigree), set as a random effect, to decompose the phenotypic variance of every response variable in additive genetic variance, i.e. the variance explained by inheritance of alleles, and the remaining variance (e.g. environmental effects). Heritability is the degree of phenotypic variation that is due to genetic inheritance between generations in a population and is calculated as the ratio (limited between 0 and 1) of the additive genetic variance to the total phenotypic variance (de Villemereuil 2018). Since our aim is to assess heritability of host quality measured through tick performance, phenotypic data of ticks were linked to the bird pedigree in our animal models. Four chains were ran in parallel in the 'brms' package with default weakly informative priors. We specified a binomial (logit link) conditional distribution of the response variable for the success ratios (attachment, feeding, moulting and survival success) and a Gaussian (identity link) distribution for the other variables. Feeding and moulting time for both larvae and nymphs were log-transformed to normalize their distributions. For binomial distributions the variance

of the standard logistic distribution (equal to $\pi^2/3$) was accounted for in the estimate of the total phenotypic variance (Davies et al. 2015, de Villemereuil 2018). Bird pedigree and batch were fitted as random effects while no fixed effect was specified. Model convergence and autocorrelation was checked using the diagnostic functions embedded in the ‘brms’ package to analyse the posterior distributions, chain autocorrelations and efficiency of the sampler. An heritability estimate was considered consistently different from zero when the shape of its posterior distribution approached a Gaussian distribution. The plots of the posterior distributions and estimates of the additive genetic and residual variance are shown in the Supporting information.

Host traits and feeding performance

In separate generalized linear mixed effects models we investigated the effect of host body condition at capture, change in body condition (difference between capture and the fourth day from infestation), sex and age, on each tick performance variable. We fitted a binomial distribution (logit link) for models on tick success ratios and a Gaussian distribution (identity link) for the other tick variables in the ‘lme4’ (ver. 1.1-26) package (Bates et al. 2015). To normalize the variables, nymph feeding time and moulting time (larvae and nymphs) were log-transformed while we applied a square-root transformation to larval feeding time. Host traits (i.e. body condition at capture, change in body condition, sex, age) were set as fixed effects while host ID and batch were set as random effects. Tick fasting time and feeding density were included as covariates since studies on the same tick species showed that these covariates can affect tick performance (Fracasso et al. 2022, Van Oosten et al. 2016). However, since feeding density was largely determined by attachment rate, we excluded it from the models on attachment success, and also from the model on survival as it already included the variation in attachment. The same models were also run on a subset of birds for which a blood sample was taken (blood-sampled subset hereinafter), in order to include individual haematocrit levels in the analyses as fixed effect. It is worth noting that the blood-sampled subset is inevitably biased with respect to body condition since we did not take a blood sample from birds with low weight, i.e. low body condition. A low host body condition could be due to several factors including (co)infection with pathogens or other parasites, however this was not investigated in the present study. Differences between the two models (i.e. with or without haematocrit) with regard to effect sizes and/or significance will be explicitly mentioned. However, signs of the significant effects never differed between the two models (Supporting information). To maximize sample size, statistical power and to account for type I errors due to multiple testing: 1) only variables with $p < 0.01$ were considered as main results though all p -values below 0.05 are reported; 2) the full models were reduced by sequentially removing the predictor with the highest p -value (backward selection) until the improvement in Akaike information criterion (AIC) of the reduced model was lower than two compared to the previous model. In all cases, variables that explained part of the variation but were

weakly significant ($0.01 < p < 0.05$) were left in the models. We started from the full models (i.e. including all host traits) as we were interested in investigating the effect of every host trait on tick performance. Multi-collinearity between explanatory variables was investigated for every model and no significant correlations were found. Interactions between fixed effects were not included to limit the number of models considered and hence the occurrence of type I errors due to multiple testing. p -values for models on success ratios were calculated on a Z-distribution while for all other tick performance variables we used the Student’s t -distribution. In the rare cases when a model ran into a convergence warning or a singular fit, we also ran an equivalent Bayesian model. In all cases the Bayesian model supported the results of the frequentist one (results not shown).

In total, we carried out 165 feeding events for larvae and 90 for nymphs for a total of 4467 larvae and 565 nymphs put on the hosts. Five feeding events where no ticks attached to the bird were excluded from the analysis on feeding and moulting success. Ten additional birds were excluded from the analysis on moulting success since we did not recover any ticks despite some of them were presumably attached after infestation. Therefore, the number of individuals and groups differ between parasite variables due to missing data at different stages of the study.

Results

Between-stage correlation and heritability

The between-stage correlation in tick performance within hosts showed a significant and moderate correlation for attachment success (estimate: 0.351 [0.018; 0.610], Table 1), thus birds with a high attachment rate for nymphs also had a high attachment rate for larvae (in a separate infestation batch), and vice versa. We found no between-stage correlations for any other tick performance variable, with estimates ranging from -0.3 to 0.4 (Table 1).

We found evidence for substantial heritability in host quality expressed in both larval and nymph performance (Table 2, Fig. 2). Specifically, in larvae feeding time had the highest heritability ($h^2 = 0.486$ [0.109; 0.826]) followed by feeding success ($h^2 = 0.162$ [0.123; 0.204]). All other larval success ratios – i.e. attachment, moulting and survival success – showed heritability to some extent (range h^2 : 0.065–0.105). In nymphs, host genetic background (bird pedigree) explained a considerable part of the variation in moulting success ($h^2 = 0.266$ [0.047; 0.497]). Although the lower 95% credible intervals (95% CI hereinafter) for the heritability of host quality for nymphal attachment, moulting and survival success approached zero, the shape of their posterior distributions strongly suggest some degree of heritability for these tick variables as well (Supporting information). On the contrary, for all other performance measures the 95% CI and the shape of their posterior distributions show that these heritability estimates were not considerably different from zero.

Table 1. Between-stage correlation of tick performance on individual hosts with the number of infested birds used to estimate each variable (N). In round brackets, birds infested with both life stages. Six birds were repeatedly exposed with nymphs and three birds with larvae. In squared brackets, 95% CI. In bold, between-stage correlations whose 95% CI do not overlap zero.

	Larvae and nymphs	N
On host		
Attachment success	0.351 [0.018; 0.610]	229 (25)
Feeding time	-0.320 [-0.628; 0.088]	190 (23)
Weight	0.440 [-0.246; 0.771]	190 (23)
Feeding success	0.087 [-0.222; 0.372]	229 (25)
Off host		
Moulting time	-0.155 [-0.543; 0.271]	190 (23)
Moulting success	-0.096 [-0.411; 0.238]	190 (23)
Overall survival	0.346 [-0.139; 0.681]	190 (23)

Attachment success: proportion of ticks presumably attached out of ticks infested.

Feeding success: proportion of recovered ticks out of presumably attached ticks.

Moulting success: proportion of moulted ticks out of ticks recovered engorged.

Overall survival: proportion of ticks put on the bird that moulted to the next stage.

Effect of host traits

Below, we report the results from the reduced models; in all cases they were similar to those of the full models (shown in the supporting information). We found that larval feeding success and overall survival were higher when larvae were put on male great tits (feeding success estimate: 0.508, SE=0.136, $z=3.74$, $p < 0.001$, Fig. 3; survival estimate: 0.426, SE=0.137, $z=3.11$, $p=0.002$, Fig. 4). Also, a higher proportion of nymphs survived on older birds (estimate: 0.202, SE=0.059, $z=3.41$, $p < 0.001$, Fig. 5) but in contrast to larvae, no effects of host sex were observed (Table 3, Fig. 6, 7). Finally, feeding time was negatively correlated with host haematocrit in larvae (estimate: -0.083, SE=0.028, $t=-3.02$, $p=0.003$).

With regard to the covariates, we found that both feeding time in larvae and moulting time in nymphs increased with fasting time (feeding time estimate: 0.109, SE=0.031, $t=3.51$, $p < 0.001$; moulting time estimate: 0.143, SE=0.050, $t=2.83$, $p=0.006$). Feeding density did not

significantly affect any tick performance variable, neither in larvae nor in nymphs.

In addition to the abovementioned correlations our results also possibly suggest ($0.01 < p\text{-value} < 0.05$) that a higher reduction in host body condition and older hosts would increase larval attachment success and nymph moulting success, respectively. Additionally, host haematocrit could correlate positively with larval moulting success and engorgement weight, and negatively with larval feeding success; it could also negatively correlate with nymph attachment success and survival. These correlations support previous findings of nymphs being more successful on older hosts and suggest a complex relationship between host haematocrit and tick performance. Lastly, fasting time may increase larval moulting time and reduce larval moulting success as well as nymph attachment and survival success (Table 3, Supporting information) suggesting an overall negative impact of starvation on the performance of larvae and nymphs. However, given the high number of tests performed (high type-I error risk) and biased sample (non-random blood-sampled birds), we conservatively avoid to consider them as biologically relevant correlations.

Discussion

In this study we provide evidence for consistent variation among individuals in host quality, as measured through various on-host and off-host performance measures of tick larvae and nymphs feeding on wild-caught great tits. Specifically, we show that attachment success of larvae and nymphs are correlated at the within-host level. Based on our findings from eight out of fourteen performance variables, we conclude that host quality is heritable. In detail, we found substantial host heritability for several measures related to host exploitation by ticks, most notably larval feeding time and feeding success. Substantial heritability was also found for success ratios of ticks related to off-host development, especially the moulting success in nymphs. The existence of differential host effects is suggested by an array of significant associations between host traits (sex, age and haematocrit) with one or more of the tick performance measures mentioned above.

Table 2. Strength of the influence of host genetic background (host heritability) on tick performance with number of infested birds used to estimate each variable (N). In round brackets, birds infested with both life stages. In squared brackets, 95% CI.

	Larvae		Nymphs	
	h^2	N	h^2	N
On host				
Attachment success	0.065 [0.033; 0.099]	164 (3)	0.041 [0.000; 0.090]	90 (6)
Feeding time	0.486 [0.109; 0.826]	123 (1)	0.170 [0.000; 0.467]	90 (6)
Weight	0.100 [0.000; 0.330]	123 (1)	0.128 [0.000; 0.403]	90 (6)
Feeding success	0.162 [0.123; 0.204]	164 (3)	0.039 [0.000; 0.099]	90 (6)
Off host				
Moulting time	0.059 [0.000; 0.218]	123 (1)	0.118 [0.000; 0.421]	90 (6)
Moulting success	0.105 [0.053; 0.157]	123 (1)	0.266 [0.047; 0.497]	90 (6)
Overall survival	0.105 [0.070; 0.144]	123 (1)	0.027 [0.000; 0.063]	90 (6)

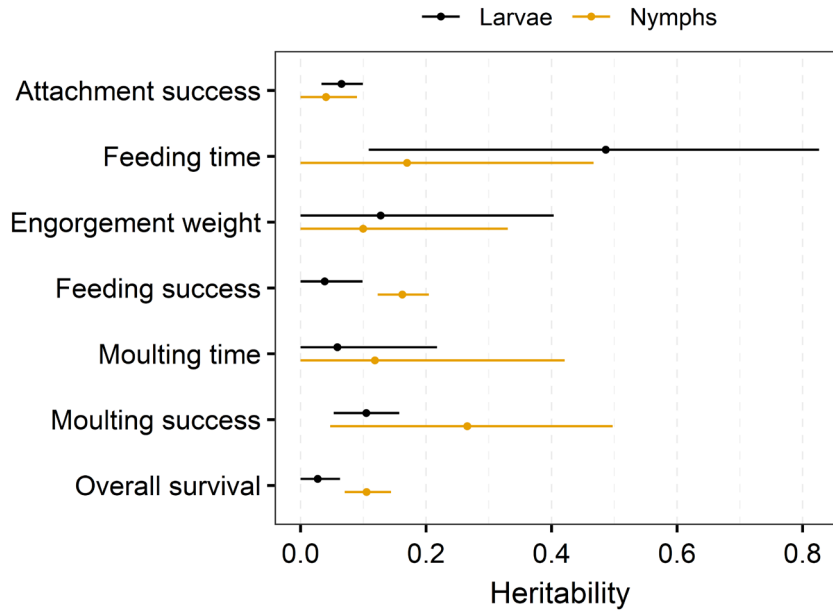


Figure 2. Strength of the influence of host genetic background (host heritability) on performance variables of larvae (black) and nymphs (yellow). Dots and horizontal lines show mean estimates and 95% credible intervals respectively.

The positive correlation in attachment success between larvae and nymphs feeding on the same bird shows that there is underlying variation in host quality that similarly affected the attachment success of immature ticks. This suggests that different parasite stages may be affected by similar selective pressures originating from the host. Furthermore, we infested hosts with larvae and nymphs at different occasions (substantially separated in time) and hence the positive correlation was maintained irrespective of temporal variation. Positive between-stage correlations of tick loads within individual hosts were also found in wild populations of sleepy lizards (Payne et al. 2020) although this could be due to differences in parasite exposure. To the best of our knowledge, no other study investigated within-host correlations of

tick performance variables between life stages. Although it is known that host individuals are affected by parasitism to varying degrees in a population (Combes 2001), it is unclear to what extent hosts vary between them in quality from the parasite point of view, and how this may vary throughout time and across parasite stages. The identification of hosts that mostly contribute to parasite transmission is key to design targeted, less expensive and efficient programs for disease control (Perkins et al. 2003). Attachment success can result from the combination of both host characteristics and tick choice. On the one hand, hosts can defend themselves through resistance mechanisms such as behavioural defence, e.g. grooming (Bush and Clayton 2018) or via integumentary

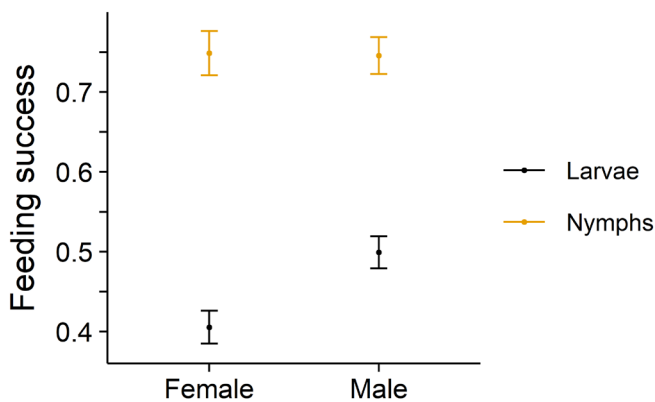


Figure 3. Mean observed feeding success (proportion of recovered ticks out of attached ones) in male and female great tits for larvae (black) and nymphs (yellow). Error bars represent ± 1 standard error of the mean. In larvae, sex differences were statistically significant in the respective GLMM.

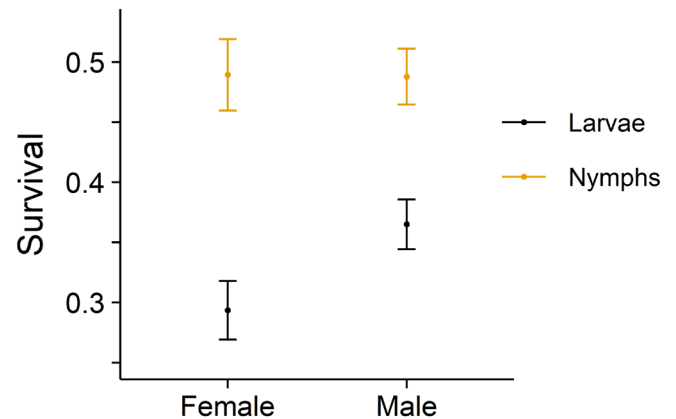


Figure 4. Mean observed overall survival (from infestation to moulting into the next stage) in male and female great tits for larvae (black) and nymphs (yellow). Error bars represent ± 1 standard error of the mean. In larvae, sex differences were statistically significant in the respective GLMM.

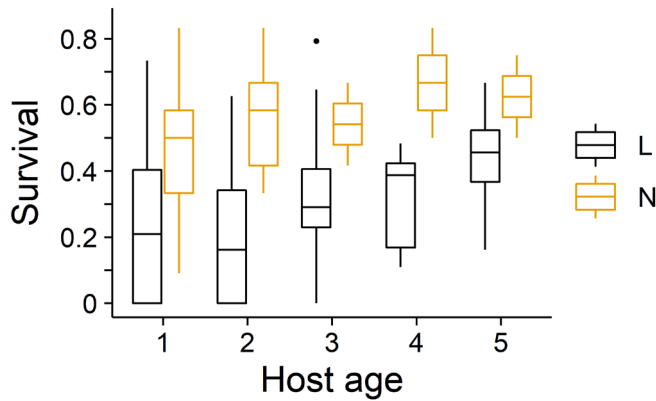


Figure 5. Mean observed overall survival (from infestation to moulting into the next stage) of larvae (L, black) and nymphs (N, yellow) put on great tits of different age (in years). Median (horizontal lines), interquartile range (box limits) and potential outliers (dots) for every age class are shown. The only six-year old host is not shown for visual clarity. In nymphs, age differences were statistically significant in the respective GLMM.

properties (e.g. skin thickness and feather/fur density) hindering or preventing tick attachment (Owen et al. 2009). On the other hand, hosts are a resource for the parasite and as such they could differ in attractiveness. Ticks heavily rely on the assessment of chemical compounds, including while seeking for hosts (Sonenshine 2004, Sonenshine and Roe 2013). In this context, hosts attractiveness might be conveyed by the specific blend of chemical volatiles emitted by a host, namely

its olfactory signature (Bonadonna et al. 2007, Hagelin and Jones 2007). In the latter case, it could be hypothesized that attachment success is a proxy of overall host quality if host defence is assumed constant between hosts. However, this hypothesis is unlikely as we found no correlation between attachment and feeding success within hosts for both larvae and nymphs (results not shown). Our results on the significant effect of host identity for tick attachment success are in line with Heylen and colleagues (2013) where the identity of free-living great tits explained part of the variation in the infestation levels of *I. ricinus* ticks. In our experimental study we succeeded to completely rule out extrinsic sources of variability, which is hard to do in the wild populations. In fact, the birds were exposed to an equal number of ticks in a very standardized environment. Also field studies on wild host populations found repeatable tick infestation levels at the level of host individuals, e.g. great tits (Heylen et al. 2013) and white-footed mice (Devevey and Brisson 2012) where hosts are naturally exposed to exophilic ticks (*I. ricinus* and *Ixodes scapularis*, respectively) living in the understory vegetation. Whether those are due to habitat use or intrinsic host quality, remains a question.

We found evidence for substantial heritability of host quality, expressed in various measures related to tick performance. The effect of host genetic background, namely the heritability of host quality, was greatest for larval feeding time. This variable is not only a measure of how quickly ticks can overcome host defence and acquire resources, it also reflects the choice of leaving the host (detachment) once a

Table 3. Estimated effects of host traits, tick fasting time and feeding density on tick performance (on- and off-host): success ratios (binomial, logit link) and continuous variables (Gaussian, identity link). Estimates refer to the most reduced model (backward stepwise selection) with the highest sample size for the predictor. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001. *Difference in significance respect to the model with haematocrit. ^aBlood-sampled subset.

	Host					Tick	
	BC	BC change	Sex	Age	Hct ^a	Fasting	Density
Larvae							
On host							
Attachment success	-0.046	0.199*	0.080	-0.028	-0.009	0.129	-
Feeding time	-0.023	0.010	-0.052	-0.022	-0.083**	0.109***	-0.001
Weight	-0.440	0.566 [†]	0.719 [†]	0.240	0.583*	-0.144	0.034
Feeding success	-0.025	0.098	0.508***	-0.032	-0.219*	-0.042	0.002
Off host							
Moulting time	0.031	-0.023	-0.029	0.015	0.010	0.099*	-0.005
Moulting success	0.014	0.104	0.006	0.157	0.232*	-0.288*	0.012
Overall survival	-0.047	0.059	0.426**	-0.008	-0.022	-0.090	-
Nymphs							
On host							
Attachment success	-0.143	0.065	-0.074	0.138	-0.237*	-0.230*	-
Feeding time	0.039	0.023	0.030	-0.071	0.037	0.027	0.020 [†]
Weight	-0.086	0.073	-0.220	0.087	-0.128	-0.107	0.008
Feeding success	0.080	0.112	-0.180	0.111	-0.138	-0.145	0.020
Off host							
Moulting time	0.031	0.057	0.168	-0.079 [†]	0.009	0.143**	0.029 [†]
Moulting success	0.176	-0.047	-0.145	0.806*	-0.181	-0.146	-0.051
Overall survival	-0.008	0.076	-0.166	0.202***	-0.193*	-0.195**	-

BC: host body condition (scaled-mass index) at capture.

BC change: change in host body condition between capture and peak of tick detachment.

[†] variable not included in the model.

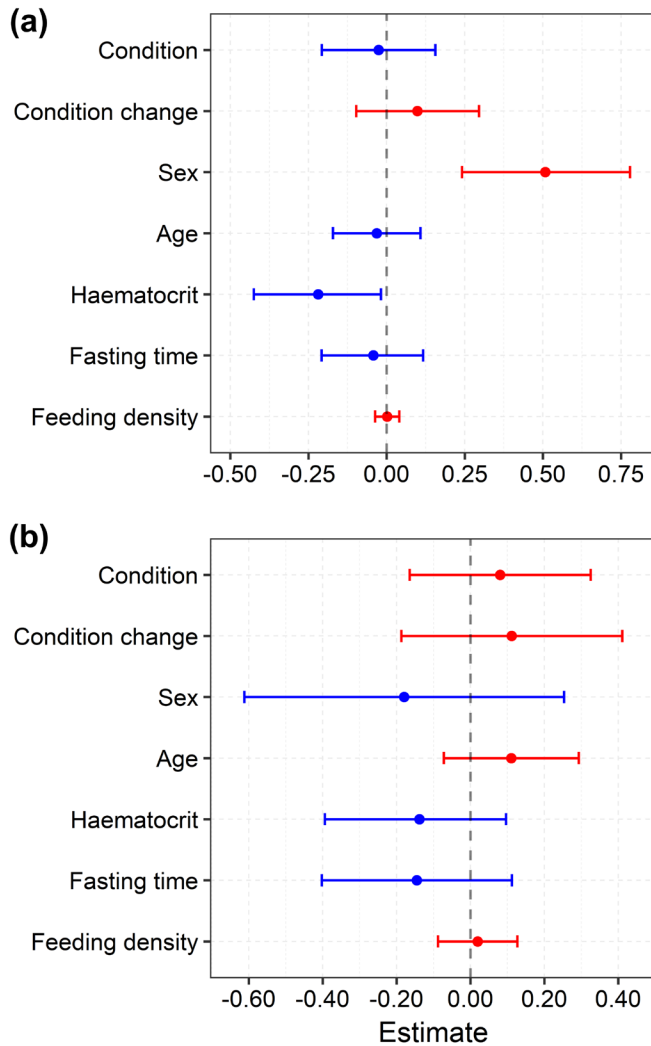


Figure 6. Mean effect size and 95% confidence intervals of host traits on feeding success (proportion of recovered ticks out of attached ones) of larvae (a) and nymphs (b). Positive estimates are in shown in red, negative estimates in blue.

suitable habitat is found (White et al. 2012). As such, feeding time also affects tick dispersal. A study on the same dataset showed that tick feeding time has low evolutionary potential and that it is substantially affected by host identity (Ancillary manuscript). Unmeasured host traits might underlie the causal mechanisms explaining the high heritability for feeding time, such as variation in skin thickness which has been found to be heritable in other birds (Deng et al. 2020). This kind of variation might also explain why between-host variation had a larger effect on larvae as they have a much smaller feeding organ (hypostome) compared to nymphs.

Although relatively low, our estimates show evidence of evolutionary potential in host characteristics that are associated with both susceptibility to parasite infestation (attachment and feeding success) and parasite survival to the next stage (moulting success and overall survival). This can have important evolutionary consequences as heritable variation

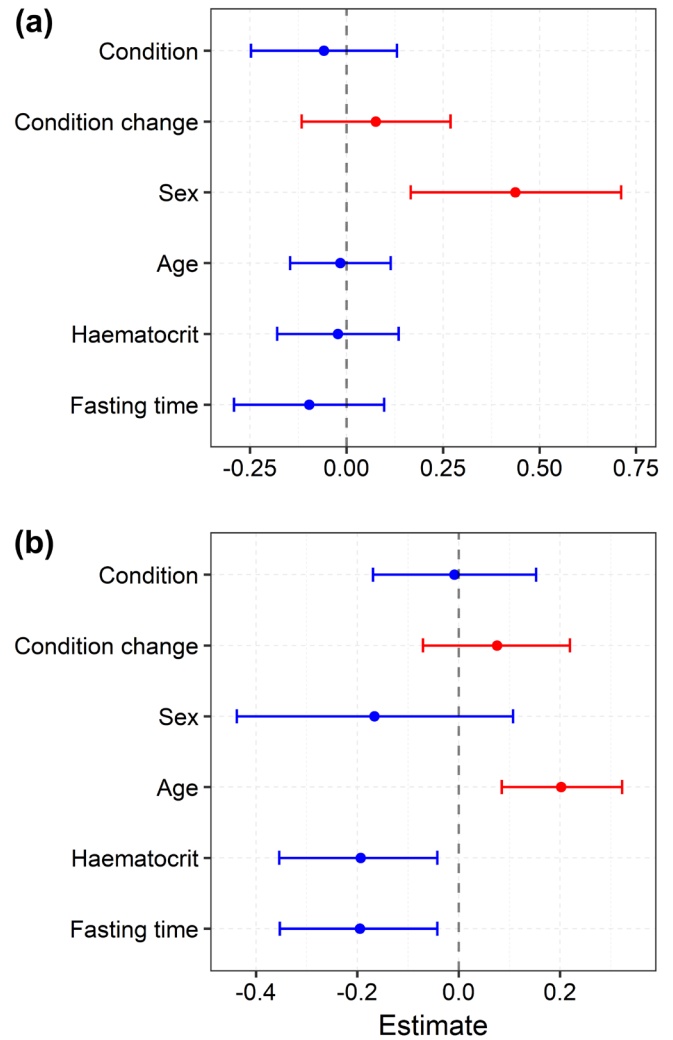


Figure 7. Mean effect size and 95% confidence intervals of host traits on overall survival (from infestation to moulting into the next stage) of larvae (a) and nymphs (b). Positive estimates are in shown in red, negative estimates in blue.

is an essential element for host–parasite coevolution. To our knowledge, this is one of the first studies showing heritability in host quality with respect to multiple ectoparasite performance measures in wild animal populations (Mazé-Guilmo et al. 2014, Saura et al. 2019, Stutz et al. 2019). Interestingly, host quality tended to have a slightly lower heritability for nymphs. Further research is needed to assess the consistency of the latter result and extend it to other multi-stage ectoparasites.

Several hosts traits significantly correlated with parasite performance. Larvae had lower feeding and survival success on great tit females. Sex-related differences in parasite intensity and prevalence, in particular female hosts being less susceptible, are a well-known pattern in host–parasite interactions (Tschirren et al. 2003, Roberts et al. 2004, Ruiz-Fons et al. 2013). We hypothesize that male hosts may have less effective, or more costly, defence mechanisms compared to females. For instance, the high testosterone levels typically found in males

have been shown to reduce both cell-mediated and humoral immunity and to be linked with ectoparasite load (Duffy et al. 2000, Poiani et al. 2000, Klukowski and Nelson 2001). Sex differences in host grooming or in other physiological measures could also play a role (Cotgreave and Clayton 1994). Higher tick loads have been previously showed in a congeneric species, *Ixodes ricinus*, on male versus female great tits (Heylen et al. 2013). Our study indicates that at least part of this variation may be related to intrinsic host quality, and not only be due to tick exposure such as through differences in foraging activity (hypothesized by Heylen et al. 2009).

Nymph survival was higher on older hosts, as has been found in ticks feeding on deer mice (Jones et al. 2015). More generally, very few empirical studies have examined host age effects on parasite performance (Lawrence et al. 1976, Izhar and Ben-Ami 2015, Izhar et al. 2015) and the causal mechanisms at the base of this relationship remain unclear.

Larval feeding time was negatively correlated with host haematocrit. High haematocrit means a high concentration of erythrocytes per unit volume. Hence, ticks engorging on hosts with high haematocrit might need less time to ingest a proper amount of energy resources. Bird haematocrit has previously been shown to be partially heritable but estimates vary strongly between studies and species (Shlosberg et al. 1998, Potti et al. 1999, Christe et al. 2000, Fair et al. 2007), and other factors such as season, sex and age have also been shown to play a role (Norte et al. 2009, Pap et al. 2010). Bird body condition at capture, i.e. two days prior to infestation, did not affect tick performance. Host body condition can have contrasting effects on parasite performance depending on whether it mainly enhances host immunity (reviewed in Wakelin 1989) or increases the resources available for the parasite (Bedhomme et al. 2004, Seppälä et al. 2008). Our results support a meta-analysis of a wide range of host-parasite systems (Pike et al. 2019) showing an overall lack of correlation between host nutrition and parasite virulence. However, in our study the acclimation time and differences in bird response to the indoor environment might have contributed to neutralize any effect of initial body condition on parasite performance. Also, it cannot be excluded that bird infection with other (micro)parasites may have affected tick performance through a reduction in host body condition. For instance, *I. ricinus* nymphs have been shown to prefer feeding on *Borrelia*-infected bank voles, and infected nymphs had a higher body weight (van Duijvendijk et al. 2017). There is also evidence that tick-borne pathogens can modify tick behaviour potentially altering tick performance (Benelli 2020).

In line with our expectations, we found that tick fasting time affected tick performance and should thus be taken into account in future studies on multi-stage parasites. Specifically, larvae fed longer and nymphs took longer to moult when more time had elapsed since the previous bloodmeal. Also in other acary, longer fasting times have been linked to the reduced feeding success in larvae of Rocky Mountain wood ticks (Jones et al. 2015) and with the reduced likelihood to initiate parasitism in the mite *Arrenurus planus* (Robb and Forbes 2005).

In conclusion, we found that the attachment success of larvae and nymphs is positively correlated within hosts irrespective of temporal variation. Furthermore, host genetic background significantly affected multiple aspects of tick performance thus suggesting heritable variation in host quality. Such heritability is a fundamental condition to allow host-parasite coevolution to occur. We also identified some host traits that explained host quality, possibly in an indirect way. Our findings point out that even within the same species, not all hosts have equal value for the parasite. Moreover, larval and nymph performance appear to be often affected by different host characteristics. Similar differences between developmental stages could be evident in other ectoparasite species as well. Hence, we suggest prudence in generalizing stage-specific findings. It is particularly remarkable that such intrinsic host variability is present in a host seemingly unable to mount an effective immune response against the parasite (Heylen et al. 2010, 2021). Even though the underlying mechanisms remain unknown, correlations between host traits and parasite performance can strongly affect parasite population dynamics and disease spread both within and between species (Ostfeld and Keesing 2000, Lloyd-Smith et al. 2005, VanderWaal and Ezenwa 2016). Heterogeneity in host quality may be widespread in host-parasite systems with important ecological and evolutionary consequences on populations and communities. We therefore suggest that more research focusing on the parasite perspective will be greatly beneficial for the comprehension of host-parasite interactions.

Acknowledgements – We want to thank Joris Elst, Sophie Philtjens and Elena Eisenring for assistance during data collection as well as Stefan Van Dongen for statistical advice.

Funding – This work was supported by an FWO (Fonds voor Wetenschappelijk Onderzoek – Flanders, Belgium) project (grant number G.0538.17) awarded to EM and DH. DH is funded by the Marie Skłodowska-Curie Actions, European Union (EU-Horizon 2020, Individual Global Fellowship, project no. 799609). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

Gerardo Fracasso: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Writing – original draft (lead); Writing – review and editing (equal). **Erik Matthysen:** Conceptualization (equal); Funding acquisition (equal); Methodology (supporting); Project administration (equal); Resources (lead); Supervision (lead); Validation (equal); Writing – review and editing (equal). **Dieter Heylen:** Conceptualization (equal); Formal analysis (supporting); Funding acquisition (equal); Methodology (supporting); Project administration (supporting); Supervision (equal); Validation (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.2547d7wrt>> (Fracasso et al. 2021).

Supporting information

The supporting information associated with this article is available from the online version.

References

- Barrett, L. G. et al. 2008. Life history determines genetic structure and evolutionary potential of host–parasite interactions. – *Trends Ecol. Evol.* 23: 678–685.
- Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. – *J. Stat. Softw.* 67: 48.
- Bedhomme, S. et al. 2004. Virulence reaction norms across a food gradient. – *Proc. R. Soc. B* 271: 739–744.
- Benelli, G. 2020. Pathogens manipulating tick behavior – through a glass, darkly. – *Pathogens* 9: 664.
- Best, A. et al. 2009. The implications of coevolutionary dynamics to host–parasite interactions. – *Am. Nat.* 173: 779–791.
- Birchard, G. F. 1997. Optimal hematocrit: theory, regulation and implications. – *Am. Zool.* 37: 65–72.
- Bize, P. et al. 2008. What makes a host profitable? Parasites balance host nutritive resources against immunity. – *Am. Nat.* 171: 107–118.
- Bonadonna, F. et al. 2007. Individual odor recognition in birds: an endogenous olfactory signature on petrels' feathers? – *J. Chem. Ecol.* 33: 1819–1829.
- Bowers, E. K. et al. 2014. Neonatal body condition, immune responsiveness and hematocrit predict longevity in a wild bird population. – *Ecology* 95: 3027–3034.
- Bürkner, P.-C. 2017. Brms: an R package for Bayesian multilevel models using Stan. – *J. Stat. Softw.* 80: 1–28.
- Bürkner, P.-C. 2018. Advanced Bayesian multilevel modeling with the R package brms. – *R J.* 10: 395–411.
- Bush, S. E. and Clayton, D. H. 2018. Anti-parasite behaviour of birds. – *Phil. Trans. R. Soc. B* 373: 20170196.
- Bush, S. E. et al. 2019. Host defense triggers rapid adaptive radiation in experimentally evolving parasites. – *Evol. Lett.* 3: 120–128.
- Carval, D. and Ferriere, R. 2010. A unified model for the coevolution of resistance, tolerance and virulence. – *Evolution* 64: 2988–3009.
- Christe, P. et al. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). – *Heredity* 85: 75–83.
- Christe, P. et al. 2003. Differential species-specific ectoparasitic mite intensities in two intimately coexisting sibling bat species: resource-mediated host attractiveness or parasite specialization? – *J. Anim. Ecol.* 72: 866–872.
- Christe, P. et al. 2007. Host sex and ectoparasites choice: preference for, and higher survival on female hosts. – *J. Anim. Ecol.* 76: 703–710.
- Clayton, D. H. and Moore, J. 1997. Host–parasite evolution: general principles and avian models. – Oxford Univ. Press.
- Clayton, D. H. et al. 2005. Adaptive significance of avian beak morphology for ectoparasite control. – *Proc. R. Soc. B* 272: 811–817.
- Clayton, D. H. et al. 2015. Coevolution of life on hosts: integrating ecology and history. – Univ. of Chicago Press.
- Clutton-Brock, T. and Sheldon, B. C. 2010. Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. – *Trends Ecol. Evol.* 25: 562–573.
- Combes, C. 2001. Parasitism. The ecology and evolution of intimate interactions. – Univ. of Chicago Press.
- Cornet, S. et al. 2014. Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. – *J. Anim. Ecol.* 83: 256–265.
- Cotgreave, P. and Clayton, D. H. 1994. Comparative analysis of time spent grooming by birds in relation to parasite load. – *Behaviour* 131: 171–187.
- Cramp, S. and Perrins, C. 1993. Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic: flycatchers to shrikes. – Oxford Univ. Press.
- Davies, S. W. et al. 2015. Estimating trait heritability in highly fecund species. – *G3* 5: 2639–2645.
- de Villemereuil, P. 2018. Quantitative genetic methods depending on the nature of the phenotypic trait. – *Ann. N. Y. Acad. Sci.* 1422: 29–47.
- Deng, M.-T. et al. 2020. Genome-wide association study reveals novel loci associated with fat-deposition and meat-quality traits in Pekin ducks. – *Anim. Genet.* 51: 953–957.
- Devevey, G. and Brisson, D. 2012. The effect of spatial heterogeneity on the aggregation of ticks on white-footed mice. – *Parasitology* 139: 915–925.
- Đlugosz, E. M. et al. 2014. Ectoparasite performance when feeding on reproducing mammalian females: an unexpected decrease when on pregnant hosts. – *J. Exp. Biol.* 217: 1058–1064.
- Dube, W. C. et al. 2018. Microclimate and host body condition influence mite population growth in a wild bird-ectoparasite system. – *Int. J. Parasitol.* 7: 301–308.
- Duffy, D. L. et al. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. – *Behav. Ecol.* 11: 654–662.
- Fair, J. et al. 2007. Sources of variation in haematocrit in birds. – *Ibis* 149: 535–552.
- Firth, J. A. et al. 2015. The influence of nonrandom extra-pair paternity on heritability estimates derived from wild pedigrees. – *Evolution* 69: 1336–1344.
- Fracasso, G. et al. 2019. Experimental study of micro-habitat selection by ixodid ticks feeding on avian hosts. – *Int. J. Parasitol.* 49: 1005–1014.
- Fracasso, G. et al. 2021. Data from: Heritable variation in host quality as measured through an ectoparasite's performance. – Dryad Digital Repository, <<https://doi.org/10.5061/dryad.2547d7wrt>>.
- Fracasso, G. et al. 2022. Predictors of individual performance and evolutionary potential of life-history traits in a hematophagous ectoparasite" with the following authors. – *Evolution* in press.
- Gagneux, S. 2012. Host–pathogen coevolution in human tuberculosis. – *Phil. Trans. R. Soc. B* 367: 850–859.
- Hagelin, J. C. and Jones, I. L. 2007. Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? – *Auk* 124: 741–761.
- Hart, B. L. and Hart, L. A. 2018. How mammals stay healthy in nature: the evolution of behaviours to avoid parasites and pathogens. – *Phil. Trans. R. Soc. B* 373: 20170205.
- Hartig, F. 2020. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. – R package ver. 0.3.3.0.

- <<https://cran.r-project.org/web/packages/DHARMA/index.0html>>.
- Hemingway, J. et al. 2016. Averting a malaria disaster: will insecticide resistance derail malaria control? – *Lancet* 387: 1785–1788.
- Heylen, D. and Matthysen, E. 2010. Contrasting detachment strategies in two congeneric ticks (Ixodidae) parasitizing the same songbird. – *Parasitology* 137: 661–667.
- Heylen, D. and Matthysen, E. 2011a. Experimental evidence for host preference in a tick parasitizing songbird nestlings. – *Oikos* 120: 1209–1216.
- Heylen, D. and Matthysen, E. 2011b. Differential virulence in two congeneric ticks infesting songbird nestlings. – *Parasitology* 138: 1011–1021.
- Heylen, D. et al. 2009. Offspring quality and tick infestation load in brood rearing great tits *Parus major*. – *Oikos* 118: 1499–1506.
- Heylen, D. et al. 2010. Lack of resistance against the tick *Ixodes ricinus* in two related passerine bird species. – *Int. J. Parasitol.* 40: 183–191.
- Heylen, D. et al. 2013. Ecological factors that determine *Ixodes ricinus* tick burdens in the great tit *Parus major*, an avian reservoir of *Borrelia burgdorferi* s.l. – *Int. J. Parasitol.* 43: 603–611.
- Heylen, D. et al. 2014a. Are the specialized bird ticks, *Ixodes arboricola* and *I. frontalis*, competent vectors for *Borrelia burgdorferi* sensu lato? – *Environ. Microbiol.* 16: 1081–1089.
- Heylen, D. et al. 2014b. Seasonal feeding activity of the tree-hole tick, *Ixodes arboricola*. – *Parasitology* 141: 1044–1051.
- Heylen, D. et al. 2017. Bridging of cryptic *Borrelia* cycles in European songbirds. – *Environ. Microbiol.* 19: 1857–1867.
- Heylen, D. et al. 2021. Ineffective humoral anti-tick IgY-response in birds: reaction against pathogen constituents? [ver. 2; peer review: 1 approved, 1 approved with reservations]. – *Open Research Europe*.
- Izhar, R. and Ben-Ami, F. 2015. Host age modulates parasite infectivity, virulence and reproduction. – *J. Anim. Ecol.* 84: 1018–1028.
- Izhar, R. et al. 2015. Host age modulates within-host parasite competition. – *Biol. Lett.* 11: 20150131.
- Jo, E.-K. 2019. Interplay between host and pathogen: immune defense and beyond. – *Exp. Mol. Med.* 51: 1–3.
- Jones, C. R. et al. 2015. Factors affecting larval tick feeding success: host, density and time. – *Parasit. Vectors* 8: 340.
- Karasuyama, H. et al. 2020. Immunobiology of acquired resistance to ticks. – *Front. Immunol.* 11: 601504.
- Klukowski, M. and Nelson, C. E. 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. – *Behav. Ecol. Sociobiol.* 49: 289–295.
- Korsten, P. et al. 2013. Genetic integration of local dispersal and exploratory behaviour in a wild bird. – *Nat. Commun.* 4: 2362.
- Krams, I. et al. 2010. Fattening strategies of wintering great tits support the optimal body mass hypothesis under conditions of extremely low ambient temperature. – *Funct. Ecol.* 24: 172–177.
- Lawrence, P. O. et al. 1976. Effect of host age on development of *Biostes (= Opius) longicaudatus*, a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. – *Fla. Entomol.* 59: 33–39.
- Lloyd-Smith, J. O. et al. 2005. Superspreading and the effect of individual variation on disease emergence. – *Nature* 438: 355–359.
- Lourenço, S. and Palmeirim, J. M. 2008. Which factors regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats? – *Parasitol. Res.* 104: 127.
- Masri, L. et al. 2013. Sex differences in host defence interfere with parasite-mediated selection for outcrossing during host–parasite coevolution. – *Ecol. Lett.* 16: 461–468.
- Matthysen, E. 2002. Boundary effects on dispersal between habitat patches by forest birds (*Parus major*, *P. caeruleus*). – *Landscape Ecol.* 17: 509–515.
- Mazé-Guilmo, E. et al. 2014. Heritable variation in host tolerance and resistance inferred from a wild host–parasite system. – *Proc. R. Soc. B* 281: 20132567.
- Minias, P. 2020. Ecology and evolution of blood oxygen-carrying capacity in birds. – *Am. Nat.* 195: 788–801.
- Naef-Daenzer, B. et al. 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. – *J. Anim. Ecol.* 70: 730–738.
- Nauen, R. 2007. Insecticide resistance in disease vectors of public health importance. – *Pest Manage. Sci.* 63: 628–633.
- Navarro, C. et al. 2003. Dynamics of an immune response in house sparrows *Passer domesticus* in relation to time of day, body condition and blood parasite infection. – *Oikos* 101: 291–298.
- Norte, A. C. et al. 2009. Variation of adult great tit *Parus major* body condition and blood parameters in relation to sex, age, year and season. – *J. Ornithol.* 150: 651.
- Ostfeld, R. S. and Keesing, F. 2000. Biodiversity and disease risk: the case of Lyme disease. – *Conserv. Biol.* 14: 722–728.
- Owen, J. P. et al. 2009. Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite *Ornithonyssus sylviarum*. – *Int. J. Parasitol.* 39: 789–799.
- Pap, P. L. et al. 2010. Variation in haematological indices and immune function during the annual cycle in the great tit *Parus major*. – *Ardea* 98: 105–112.
- Paplauskas, S. et al. 2021. Ecology directs host–parasite coevolutionary trajectories across *Daphnia*–microparasite populations. – *Nat. Ecol. Evol.* 5: 480–486.
- Payne, E. et al. 2020. Consistent individual differences in ectoparasitism of a long-lived lizard host. – *Oikos* 129: 1061–1071.
- Peig, J. and Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. – *Oikos* 118: 1883–1891.
- Peig, J. and Green, A. J. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. – *Funct. Ecol.* 24: 1323–1332.
- Perkins, S. E. et al. 2003. Empirical evidence for key hosts in persistence of a tick-borne disease. – *Int. J. Parasitol.* 33: 909–917.
- Peterson, R. A. and Cavanaugh, J. E. 2019. Ordered quantile normalization: a semiparametric transformation built for the cross-validation era. – *J. Appl. Stat.* 47: 2312–2327.
- Pike, V. L. et al. 2019. On the diverse and opposing effects of nutrition on pathogen virulence. – *Proc. R. Soc. B* 286: 20191220.
- Poiani, A. et al. 2000. Ectoparasites of house sparrows *Passer domesticus*: an experimental test of the immunocompetence handicap hypothesis and a new model. – *Behav. Ecol. Sociobiol.* 47: 230–242.
- Potti, J. et al. 1999. Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*. – *Oecologia* 120: 1–8.
- Poulin, R. 2007. Evolutionary ecology of parasites. – Princeton Univ. Press.
- Råberg, L. et al. 2009. Decomposing health: tolerance and resistance to parasites in animals. – *Phil. Trans. R. Soc. B* 364: 37–49.
- Robb, T. and Forbes, M. R. 2005. Success of ectoparasites: how important is timing of host contact? – *Biol. Lett.* 1: 118–120.

- Roberts, K. E. and Hughes, W. O. H. 2015. Horizontal transmission of a parasite is influenced by infected host phenotype and density. – *Parasitology* 142: 395–405.
- Roberts, M. L. et al. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. – *Anim. Behav.* 68: 227–239.
- Ruiz-Fons, F. et al. 2013. Sex-biased differences in the effects of host individual, host population and environmental traits driving tick parasitism in red deer. – *Front. Cell. Infect. Microbiol.* 3: 23.
- Sanchez, A. et al. 2011. Female-biased infection and transmission of the gastrointestinal nematode *Trichuris arvicolae* infecting the common vole, *Microtus arvalis*. – *Int. J. Parasitol.* 41: 1397–1402.
- Sarabian, C. et al. 2018. Evolution of pathogen and parasite avoidance behaviours. – *Phil. Trans. R. Soc. B* 373: 20170256.
- Saura, M. et al. 2019. Disentangling genetic variation for resistance and endurance to scuticociliatosis in turbot using pedigree and genomic information. – *Front. Genet.* 10: 539.
- Seppälä, O. et al. 2008. Host condition as a constraint for parasite reproduction. – *Oikos* 117: 749–753.
- Shlosberg, A. et al. 1998. Relationship between broiler chicken haematocrit-selected parents and their progeny, with regard to haematocrit, mortality from ascites and bodyweight. – *Res. Vet. Sci.* 64: 105–109.
- Smith, J. A. et al. 1999. Heritable variation in resistance to gastrointestinal nematodes in an unmanaged mammal population. – *Proc. R. Soc. B* 266: 1283–1290.
- Sonenshine, D. E. 2004. Pheromones and other semiochemicals of ticks and their use in tick control. – *Parasitology* 129: S405–S425.
- Sonenshine, D. E. and Roe, R. M. 2013. *Biology of ticks*. – Oxford Univ. Press.
- Stutz, W. E. et al. 2019. Resistance and tolerance: a hierarchical framework to compare individual versus family-level host contributions in an experimental amphibian-trematode system. – *Exp. Parasitol.* 199: 80–91.
- Taylor, P. J. and Hurd, H. 2001. The influence of host haematocrit on the blood feeding success of *Anopheles stephensi*: implications for enhanced malaria transmission. – *Parasitology* 122: 491–496.
- Tschirren, B. et al. 2003. Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings. – *J. Anim. Ecol.* 72: 839–845.
- Tschirren, B. et al. 2007. Host condition and host immunity affect parasite fitness in a bird–ectoparasite system. – *Funct. Ecol.* 21: 372–378.
- van Duijvendijk, G. et al. 2017. A *Borrelia afzelii* infection increases larval tick burden on *Myodes glareolus* (Rodentia: Cricetidae) and nymphal body weight of *Ixodes ricinus* (Acari: Ixodidae). – *J. Med. Entomol.* 54: 422–428.
- Van Oosten, A. R. et al. 2014a. Host specificity of a bird-specialised endophilic ectoparasite, the tree-hole tick *Ixodes arboricola*. – *Parasitol. Res.* 113: 4397–4405.
- Van Oosten, A. R. et al. 2014b. Population genetic structure of the tree-hole tick *Ixodes arboricola* (Acari: Ixodidae) at different spatial scales. – *Heredity* 113: 408–415.
- Van Oosten, A. R. et al. 2016. The more the merrier—experimental evidence for density-dependent feeding facilitation in the bird-specialised tick *Ixodes arboricola*. – *Int. J. Parasitol.* 46: 187–193.
- Van Oosten, A. R. et al. 2018. Sex ratios of the tick *Ixodes arboricola* are strongly female-biased, but there are no indications of sex-distorting bacteria. – *Ticks Tick-Borne Dis.* 9: 307–313.
- VanderWaal, K. L. and Ezenwa, V. O. 2016. Heterogeneity in pathogen transmission: mechanisms and methodology. – *Funct. Ecol.* 30: 1606–1622.
- Villa, S. M. et al. 2018. Beak of the pinch: anti-parasite traits are similar among Darwin's finch species. – *Evol. Ecol.* 32: 443–452.
- Wakelin, D. 1989. Nature and nurture: overcoming constraints on immunity. – *Parasitology* 99: S21–S35.
- White, J. et al. 2012. Adaptive timing of detachment in a tick parasitizing hole-nesting birds. – *Parasitology* 139: 264–270.
- Wilson, A. J. et al. 2010. An ecologist's guide to the animal model. – *J. Anim. Ecol.* 79: 13–26.
- Yessinou, R. E. et al. 2016. Resistance of tick *Rhipicephalus microplus* to acaricides and control strategies. – *J. Entomol. Zool. Stud.* 4: 408–414.