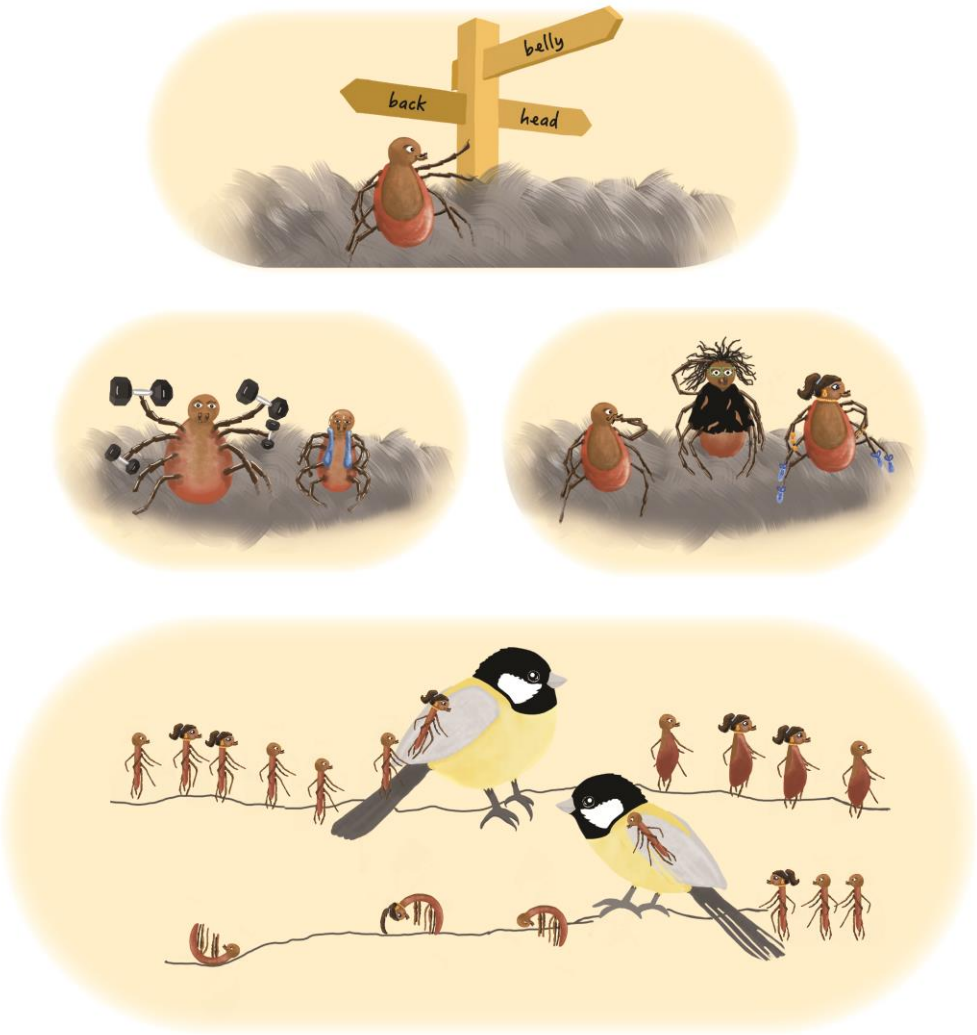
Individual variation in an ectoparasite-host system

Life history, fitness and evolutionary potential

Gerardo Fracasso



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Individual variation in an ectoparasite-host system: life history, fitness and evolutionary potential

Individuele variatie in een ectoparasiet-gastheersysteem: levensgeschiedenis, fitness en evolutionair potentieel

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Summary

Parasites are among the most ubiquitous and widespread living forms on earth exerting direct and indirect negative effects to the organisms they exploit. On their side, hosts try to reduce parasite burden through several adaptations. The dynamic and reciprocally exerted selective pressures make host-parasite interactions ideal systems for the study of coevolutionary processes and their effects on ecology. However, while the effects of parasites on host life history, defence strategies and evolution have been extensively investigated host-induced parasite evolution and life history were mainly neglected until recently. Furthermore, parasites have mostly been considered as agents of selection rather than as species in their own right. One of the most important characteristics of a species is its among- and within-individual variation in traits and overall performance as it is fundamental to respond to selective pressures (e.g. environmental variation) and generate adaptations. Nevertheless, the amount of variation for most parasite traits is unknown. Another crucial element defining the evolutionary ecology of a species is the relationship between its traits. In fact, the genetic architecture of traits and their phenotypic expression shape trade-offs, alternative life-history strategies, and trait evolutionary potential.

Ectoparasites provide an excellent study system to investigate individual variation in parasite traits and performance as they feed on the host surface and some of them are sufficiently large to be individually marked and followed throughout their life cycle. Among ectoparasites, hard ticks (Ixodidae) are a particularly interesting group as they are obligate parasites that feed once per stage on a different host. They spend a significant amount of time off the host thus being affected both by the host and environmental conditions. Moreover, ticks are considered the second main vector of diseases of medical and veterinary importance and thus the comprehension of processes shaping their life history and evolution is of fundamental significance. Also, ticks can be easily reared and fed in the laboratory.

In this dissertation, I report four experimental studies investigating several aspects of parasite individual variation in a tick-songbird system. Behavioural preferences, life-history traits and predictors of performance as well as the host effect on parasite traits are investigated in the bird-specialized tree-hole tick (*Ixodes arboricola*) feeding on one of its main hosts, the great tit (*Parus major*). First, I provide a general introduction on the state-of-the-art in parasite-host interactions and coevolution, in particular in tick-host systems (**chapter I**).

In **chapter II**, behavioural preferences for tick attachment sites on the host body are reported. Anecdotical observations suggest that hard ticks are only found on the head and neck of their avian hosts. However, it is unknown if this pattern is given by tick preference for specific body areas or by host grooming selectively removing ticks in some body areas but not others. Experiments were carried out with three tick species differing in ecology and host specificity both with and without grooming restriction. The experimental findings as well as the literature evidence suggest that ticks prefer to attach to the host head moving to this area if given the possibility with almost no variation between individuals and species. I hypothesize that this pattern is consistent throughout ixodid ticks feeding on bird species likely due to the lower risk of being killed or damaged by the host during feeding.

I then investigate how fundamental life-history traits affect individual tick success at every life stage and estimated their phenotypic and genetic correlation between and within stages as well as the trait evolutionary potential for feeding time, engorgement weight, moulting time, and number of hatched eggs (**chapter III**). Additionally, I account for the effect on the abovementioned traits of tick sex, maternal effect, host identity, time elapsed from the previous feeding event (fasting time) and batch. Ticks were individually followed for two consecutive generations and the relatedness between individuals was used in animal models (one per stage). My results suggest differences in tick individual quality, for which engorgement weight seems to be a good proxy. Furthermore, engorgement weight and moulting time show considerable evolutionary potential while feeding time seems to be more

affected by host identity. To the best of my knowledge this is the first study thoroughly investigating the predictors of parasite performance as well as variation and evolutionary potential of parasite traits. Lastly, I discuss the underestimated importance of such approach for future research.

In **chapter IV** I report a study investigating variation and heritability of host quality from the parasite perspective. More specifically, I measured to what extent host can affect tick performance and life history of larvae and nymphs both on- and off-host. Furthermore, I estimated the heritability of host quality as well as some host traits that may indirectly correlate with tick performance. I show that host individual characteristics significantly influenced larva and nymph attachment success. Additionally, hosts had a heritable effect on tick feeding time and, to a lower extent, on several other traits and success parameters. Interestingly, larvae had lower survival and feeding success on female hosts while nymph survival was higher on older birds. This is one of the first studies showing heritable differences in host quality for a parasite, hypothesized by previous studies in the wild and here supported by standardized laboratory conditions.

An extensive amount of literature shows that natural selection is often coupled with sexual selection. Thus, to fully comprehend the evolutionary dynamics of a species any process that can modify the phenotypic and genetic transmission of traits through sexual selection deserves full consideration. In this respect, *I. arboricola* is a particularly interesting species as it shows several characteristics favouring the evolution of male mate choice. In particular, in **chapter V** I hypothesize that males should prefer to mate with heavier engorged females in order to obtain a higher fitness. Surprisingly, mate choice experiments carried out in two different setups showed a lack of preference for heavier adult females. However, the results provide evidence that males may remember the mating partners they previously met and avoid them for at least a few days.

In the general discussion (**chapter VI**) I highlight how my results help to advance the state-of-the-art in tick research and host-parasite interactions more in

general. My research also points out the need to further investigate several other research topics such as the effect of variation in morphological traits directly related to feeding (hypostome shape), the drivers of host choice, the influence of the microbiome on parasite preferences and life history as well as the relative contribution of host morphological and physiological individual variation on host quality.

Samenvatting

Parasieten behoren tot de meest alomtegenwoordige en wijdverspreide levende wezens op aarde en hebben directe en indirecte negatieve gevolgen voor de organismen die zij exploiteren. De gastheren proberen vanuit hun kant de last van de parasieten te verminderen door zich aan te passen aan de parasiet. De dynamische en wederzijds uitgeoefende selectieve druk maakt van gastheer-parasiet interacties ideale systemen om co-evolutionaire processen en hun effecten op de ecologie te bestuderen. Hoewel de effecten van parasieten op de levensloop, de verdedigingsstrategieën en de evolutie van de gastheer uitgebreid zijn onderzocht, werden de door de gastheer geïnduceerde evolutie inzake de levensloop van parasieten tot voor kort grotendeels verwaarloosd. Bovendien werden parasieten meestal beschouwd als middel van selectie, eerder dan als volwaardige soorten. Één van de belangrijkste kenmerken van een soort is de variatie in eigenschappen en algemene prestaties tussen en binnen individuen, omdat dit van fundamenteel belang is om te kunnen reageren op selectieve druk (bv. variatie in het milieu) en zich aan te passen aan veranderende omstandigheden. Toch is de hoeveelheid variatie voor de meeste eigenschappen van parasieten onbekend. Een ander cruciaal element dat de evolutionaire ecologie van een soort bepaalt is de relatie tussen de eigenschappen. De genetische architectuur van de verschillende eigenschappen en hun fenotypische expressie bepalen de trade-offs, alternatieve levensgeschiedenisstrategieën en het evolutionaire potentieel van de eigenschappen.

Ectoparasieten vormen een uitstekend systeem om individuele variatie in parasietkenmerken en -prestaties te onderzoeken, aangezien zij zich voeden op de gastheer en sommige van hen groot genoeg zijn om individueel gemerkt en opgevolgd te worden gedurende hun levenscyclus. Onder de ectoparasieten vormen de harde teken (Ixodidae) een bijzonder interessante groep, omdat het obligate parasieten zijn die zich één keer per stadium op een andere gastheer voeden. Zij brengen een aanzienlijk deel van de tijd los van de gastheer door, zodat ze zowel

door de gastheer als door de omgeving worden beïnvloed. Bovendien worden teken erkend als de tweede belangrijkste vector van ziekten met medisch en veterinair belang. Het begrijpen van de processen die hun levensgeschiedenis en evolutie vormgeven is dus van fundamenteel belang. Bovendien kunnen teken gemakkelijk in het laboratorium worden gekweekt en gevoed.

In dit proefschrift rapporteer ik vier experimentele studies die verschillende aspecten van individuele variatie van parasieten in een teek-zangvogel systeem onderzoeken. Gedragsvoorkeuren, levensgeschiedeniskenmerken en voorspellers van prestatie en het gastheereffect op parasietkenmerken worden onderzocht bij de in vogels gespecialiseerde boomholte teek (*Ixodes arboricola*), die zich voedt op één van zijn belangrijkste gastheren: de koolmees (*Parus major*). Eerst geef ik een algemene inleiding over parasiet-gastheer interacties en co-evolutie, met een focus op teek-gastheersystemen (**hoofdstuk I**).

In **hoofdstuk II** worden gedragsmatige voorkeuren voor aanhechtingsplaatsen van teken op het lichaam van de gastheer beschreven. Anekdotische waarnemingen suggereren dat harde teken alleen op de kop en nek van hun vogelgastheren worden aangetroffen. Het is echter onbekend of dit patroon het resultaat is van voorkeur van de teek voor specifieke lichaamsdelen of door verzorging van de gastheer die selectief teken verwijdert op sommige lichaamsdelen en niet op andere. Experimenten werden uitgevoerd met drie tekensoorten die verschillen in ecologie en gastheerspecificiteit, zowel met als zonder verzorgingsrestrictie voor de vogels. De experimentele bevindingen en de literatuur suggereren dat teken zich bij voorkeur vasthechten aan het hoofd van de gastheer en zich naar dit gebied verplaatsen als ze de mogelijkheid krijgen, zonder dat er enige variatie is tussen individuen en soorten. Men kan dus veronderstellen dat dit patroon consistent is bij alle ixodide teken die zich voeden op vogelsoorten, waarschijnlijk vanwege het lagere risico om gedood of beschadigd te worden door de gastheer tijdens het voeden.

Vervolgens onderzoek ik de invloed van fundamentele levensgeschiedeniskenmerken op het succes van individuele teken in elk

levensstadium, en schat ik de fenotypische en genetische correlatie tussen en binnen de verschillende levensstadia, evenals het evolutionaire potentieel van de volgende kenmerken: voedertijd, gewicht na het voeden, duur van vervellen en aantal uitgebroede eieren (**hoofdstuk III**). Bovendien houd ik rekening met het effect van het geslacht van de teek, het maternale effect, de identiteit van de gastheer, de tijd die verstreken is sinds de vorige voeding (vastentijd) en de lot op de bovengenoemde eigenschappen. Teken werden individueel gevolgd gedurende twee opeenvolgende generaties en de verwantschap tussen individuen werd gebruikt in diermodellen (één per stadium). Mijn resultaten suggereren verschillen in de individuele kwaliteit van teken, waarvoor het gewicht na de voeding een goede proxy lijkt te zijn. Bovendien vertonen het engorgement gewicht en de vervellingsperiode een aanzienlijk evolutionair potentieel, terwijl de voedertijd meer lijkt te worden beïnvloed door de identiteit van de gastheer. Voor zover ik weet is dit de eerste studie die de voorspellers van parasietprestaties en de variatie en het evolutionaire potentieel van parasietkenmerken grondig onderzoekt. Tenslotte bespreek ik het onderschatte belang van een dergelijke benadering voor toekomstig onderzoek.

In **hoofdstuk IV** rapporteer ik een studie naar de variatie en erfelijkheid van gastheerkwaliteit vanuit het perspectief van de parasiet. Meer specifiek heb ik gemeten in hoeverre de gastheer de prestaties van de teek en de levensgeschiedenis van larven en nimfen zowel on- als off-host kan beïnvloeden. Verder heb ik de erfelijkheidsgraad van gastheerkwaliteit geschat, evenals enkele gastheerkenmerken die indirect kunnen correleren met de prestatie van teken. Ik toonde aan dat individuele gastheerkenmerken een significante invloed hadden op het aanhechtingssucces van de larven en nimfen. Bovendien hadden gastheren een erfelijk effect op de voedertijd van de teek en, in mindere mate, op verschillende andere eigenschappen en succesparameters. Interessant is dat larven een lagere overleving en voedingssucces hadden bij vrouwelijke gastheren, terwijl de overleving van nimfen hoger was bij oudere vogels. Dit is één van de eerste studies die erfelijke verschillen in gastheerkwaliteit voor een parasiet aantoont. Dit fenomeen werd in

het verleden gesuggereerd door eerdere studies in het wild en hier ondersteund door gestandaardiseerde laboratoriumomstandigheden.

Een grote hoeveelheid literatuur toont aan dat natuurlijke selectie vaak gepaard gaat met seksuele selectie. Om de evolutionaire dynamiek van een soort volledig te begrijpen verdient dus elk proces dat doormiddel van seksuele selecte de fenotypische en genetische overdracht van eigenschappen kan wijzigen door seksuele selectie alle aandacht. In dat opzicht is *I. arboricola* een bijzonder interessante soort, omdat zij verschillende kenmerken vertoont die de evolutie van mannelijke partnerkeuze bevorderen. In het bijzonder stel ik in **hoofdstuk V** dat mannetjes bij voorkeur paren met zwaarder gezwollen wijfjes om een hogere fitness te verkrijgen. Verrassend genoeg bleek uit partnerkeuze-experimenten, uitgevoerd in twee verschillende opstellingen, dat er geen voorkeur bestaat voor zwaardere volwassen wijfjes. De resultaten leveren echter wel bewijs dat mannetjes zich de paringspartners die ze eerder ontmoet hebben kunnen herinneren en ze gedurende minstens enkele dagen kunnen vermijden.

In de algemene discussie (**hoofdstuk VI**) benadruk ik hoe mijn resultaten bijdragen aan de vooruitgang van het onderzoek naar teken en meer in het algemeen naar gastheer-parasiet interacties. Mijn onderzoek wijst ook op de noodzaak om verschillende andere onderwerpen verder te onderzoeken, zoals het effect van variatie in morfologische kenmerken die direct gerelateerd zijn aan voeding (vorm van de hypostoom), de drijfveren van gastheerkeuze, de invloed van het microbioom op parasietvoorkeuren en levensgeschiedenis, evenals de relatieve bijdrage van gastheermorfologie en individuele fysiologische variatie op gastheerkwaliteit.

Chapter I

General introduction

> "According to one estimate, parasites may outnumber free-living species four to one. In other words, the study of life is, for the most part, parasitology."
>
> Carl Zimmer, *Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous Creatures*.

Parasitism is one of the most successful biological relationships as shown by the massive number of parasitic species as well as by the many independent evolutionary transitions towards parasitism (Poulin and Morand 2000). The comprehension of the dynamics driving host-parasite interactions and coevolution affects several aspects of ecology and evolutionary biology and it is a fundamental step to reduce the spread of diseases of medical and veterinary importance. For instance, parasites have been shown to play a role in natural and sexual selection (Clayton and Moore 1997), to coevolve and co-speciate with their hosts (Clayton et al. 2015; Poulin 2007), and to affect host life history (Chadwick and Little 2005).

Although the term parasite is extensively used even outside the realm of biology, its definition is somewhat vague, with differences between authors and research fields (Poulin 2007). This lack of a clear-cut definition is not unique in Biology, see for instance the definition of species, and can be attributed to the extreme diversity of living forms and natural processes that are to be found on the biosphere. In this dissertation, I will use what is likely the most widely accepted definition. **A parasite is an organism (temporarily or permanently) living in or on another organism, the host, to which it causes some harm by exploiting its resources such as food and other biological necessities** (Combes 2001; Poulin 2007). Parasite detrimental (harmful) effects on hosts determine parasite virulence which can greatly differ between parasite-host systems and vary with environmental conditions. Parasite effects can vary a lot, from nearly neutral effects which we may regard as commensalism, to severe harm which may be lethal. Parasites that

regularly kill the host in the process, are labelled parasitoids. It is worth mentioning that it is in the parasite interest to maximally exploit its host while keeping it in as good health as needed. In fact, dead hosts often become unsuitable for exploitation and thus host health needs to be traded-off with the amount of resources exploited in a given period of time. A lack of measurable detrimental effects on one or more host traits does not imply that no harm is imposed by the parasite. Negative effects can in fact be expressed in traits that were not measured or be expressed later in the host's life (Ooue et al. 2017). No matter how small a negative effect a parasite has on its host, the exploitation of resources always bears a cost to the host (Combes 2001).

Since many parasites are causative agents or vectors of diseases their effects on many aspects of host biology have received considerable attention. More recently, research has extended from the study of host immunology and physiology to the wider, and sometimes more subtle, effects that parasites have on their hosts. For instance, it is being investigated how hosts behaviourally combat parasites (Bush and Clayton 2018; Sarabian et al. 2018), how parasite and host communities affect each other (Johnson et al. 2015a; Johnson et al. 2015b), and how parasites affect host evolution (Betts et al. 2018; Gibson et al. 2018). Nevertheless, the study of parasite ecology and evolution received much less attention compared to its host counterpart (Clayton et al. 2015; Poulin 2007). In the preface to his influential book "Evolutionary Ecology of Parasites" (2007) Robert Poulin states: "With the growing recognition that parasites are omnipresent agents of natural selection as well as causes of morbidity in wildlife populations, they are increasingly seen as evolutionary and ecological forces, rather than as organisms in their own right. I aim to redress the balance". Years later, this situation has only partially changed. This dissertation aims to go in this exact same direction by adding experimental data on the ecology, evolution, and life history of a hematophagous ectoparasite using a new methodological framework. In the following sections of this general introduction the main research topics related to the context of this work are briefly introduced and outlined.

Host-parasite interactions: ecology and evolution

Organisms interact with each other and with the surrounding environment. These interactions are at the root of an individual's survival and reproduction, i.e. fitness. In parasites, the habitat in which individuals live is composed, in part or completely, by the host itself. On their part, hosts try to minimize parasite exploitation through two non-mutually exclusive defense strategies: resistance and tolerance. Resistance protects the host at the expense of the parasite (e.g. by reduction of the parasite number or fitness) through host behavioural, morphological or immune adaptations. Tolerance is instead aimed at reducing the harm caused by parasites without necessarily reducing their number or fitness (Råberg et al. 2009). Both tolerance and resistance strategies imply costs that need to be traded-off with the harm caused by the parasite itself.

One of the key elements for the understanding of parasite ecology is dispersal as it determines the amount of gene flow in a population (Clobert et al. 2012; Clobert et al. 2001). In combination with natural selection, parasite dispersal shapes host-parasite coevolution allowing the encounter between different host and parasite genotypes (Thompson 2005). In multi-host parasites, dispersal occurs by means of one or more transmission events. The combined effect of all these transmissions determines overall parasite dispersal. On the contrary, single-host parasites only need one host to complete their life cycle and thus dispersal is the outcome of this single transmission. Parasites can be transmitted between unrelated hosts (horizontal transmission) or from parents to their offspring (vertical transmission). Note that for ectoparasites the term "vertical transmission" often takes the broader meaning of transmission from parents to offspring during the rearing phase (e.g. Clayton et al. 2015) rather than mother-offspring transmission within the mother's body. This extended definition will also be used in this dissertation. Some parasite species attach to the host only temporarily to feed (e.g. ticks) and then detach to moult and/or reproduce. As these non-permanent ectoparasites carry out fundamental steps of their life cycle off the host (e.g. moulting, laying eggs) they are

likely much more affected by both the biotic and abiotic environments compared to microparasites. Other ectoparasites are instead permanent, e.g. the body louse. They can be found in the abiotic environment only when they are searching for a different host, and they are thus less exposed to changing environmental conditions.

Parasites live in or on their hosts and based on that they can be divided in two big groups. Endoparasites live inside the host body and can be further divided into intercellular (e.g. helminths) and intracellular (e.g. *Rickettsia* spp., *Trypanosoma* spp.) parasites, respectively found outside and inside the cellular environment. On the contrary, ectoparasites such as fleas, ticks and mosquitoes, live and feed on the host surface. Different cues can be used by ectoparasites while searching for a host, with chemical volatiles being one of the most important (Chaisson and Hallem 2012; Sonenshine and Roe 2013; Takken and Verhulst 2013). For instance, it has been shown that many parasites from a range of different groups, including ticks and mosquitoes, use carbon dioxide to find their host (Chaisson and Hallem 2012; Van Duijvendijk et al. 2017a). In ticks, radiant heat has also been shown to play a role (Carr and Salgado 2019). The extent to which parasites rely on a set of cues may depend on their host-seeking strategy and environment. For instance, parasites spending most of their life cycle in closed environments such as nests or burrows have a reduced need to search for hosts on a long range as compared to parasites moving freely in an open and complex environment.

From an evolutionary viewpoint, host-parasite interactions are very dynamic systems in which mutual selective pressures can lead to a chain of adaptations and counter-adaptations in both the host and parasite (Fig. 1; Carius et al. 2007; Clayton et al. 2015; Poulin 2007). They are thus ideal systems for the comprehension of evolutionary processes as a whole.

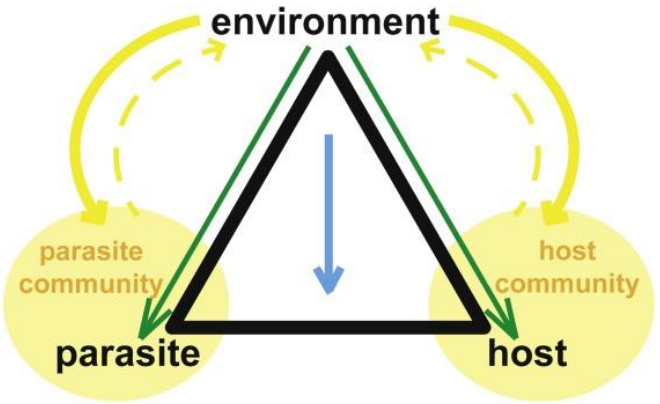


Figure 1. Schematic representation of the reciprocal interactions between parasites, hosts, and the abiotic environment. From: Brunner and Eizaguirre 2016.

Parasite variation at the individual level

Biologists have long been intrigued by the mechanisms determining individual variation. Charles Darwin himself was the first to recognize that variation between individuals was of pivotal importance for the evolution of all living organisms as it provides the raw material on which natural selection operates (Darwin 1859). More than a century later, the eminent biologist Stephen Jay Gould further emphasized this concept stating that "All evolutionary biologists know that variation itself is nature's only irreducible essence". To date, it has extensively been shown that individual variation has fundamental effects on evolutionary and ecological processes (Bolnick et al. 2011; Fogarty et al. 2011; Moran et al. 2016; Wolf and Weissing 2012). For instance, there can be important phenotypic differences between individuals within populations, and even within sex, age, or size classes (Bolnick et al. 2011; Richardson et al. 2014). Such variation has a keystone role in ecological (Des Roches et al. 2018) and evolutionary processes (Des Roches et al. 2021) but it can easily be missed at the population level due to the coarser scale. In many biological processes the relationship between a trait and the (ecological or evolutionary) response of interest is non-linear (Bolnick et al. 2011). Such mathematical relationship is called Jensen's inequality and basically states that when the relationship between two variables is nonlinear, the expectation for the response variable differs depending on whether the mean or the whole distribution of the trait is used. Results will thus be

incorrect and could lead to misleading conclusions if the entire variation and the characteristics of the relationship (e.g. linear, quadratic) are not properly taken into account (Denny 2017). Despite the importance of intraspecific variation has been recognized in several research fields such as ecology, behaviour, and physiology, research in parasitology has lagged behind. Several factors could explain this knowledge gap, of which I wish to mention the two that I believe played the biggest role. First, many evolutionary biologists are mainly interested in parasites as causative agents or vectors of diseases, or as drivers of host evolution rather than on parasites in their own right, such as on parasite ecology, life history, and evolution. Second, it is currently nearly or completely impossible to individually mark and track a substantial number of parasites that live in the host or are only found in inaccessible environments (e.g. burrows). Moreover, due to their ecology and life history many parasites cannot be kept completely separated from the host thus limiting the flexibility of experimental designs. In this context, the decoupling of the variation that is due to parasites and hosts is hampered. Also, research on parasite individual variation is further methodologically and theoretically hindered by the fact that most parasites are small, extremely prolific, often have a low survival rate, and go through multiple life stages and hosts.

The study of individual variation in parasite life-history traits and performance parameters would allow us to understand what shapes parasite fitness. In parasites, this understanding is particularly noteworthy as in many cases parasite fitness regulates disease dynamics. Importantly, the study of parasite intraspecific trait variation will also permit to measure the covariation between traits thus allowing to identify the presence of alternative life-history strategies. Last but not least, the study of individual variation in parasite traits and of the relatedness between individuals would allow to estimate the evolutionary potential of parasite traits (Barrett et al. 2008; Clayton et al. 2015; Clayton and Moore 1997). It's here worth noting that the increasing availability of genetic tools allows to establish the relatedness between individuals without any prior knowledge regarding the

population. Specifically, the evolutionary potential is the capacity of a biological entity (e.g. trait, genome, species) to exhibit heritable change in response to selection pressures (Le Rouzic and Carlborg 2008; Milot et al. 2020). At a higher level, this knowledge would permit to increase our understanding of host-parasite coevolution where adaptations and counter-adaptations can be rapid and dynamic (Carius et al. 2007; Clayton and Moore 1997; Poulin 2007; Sorci et al. 1997). Importantly, while a considerable amount of literature can be found on the variability and heritability of host traits – including those associated with tolerance and resistance (Boulinier et al. 1997; Hill 1998; Williamson and Kumar 2006; Kause et al. 2012; Mazé-Guilmo et al. 2014; Ayres et al. 2015) – knowledge on the variability and heritability of parasite traits is currently lacking for most parasites. This is likely due to the prevailing theoretical approach and methodological difficulties in parasitology and evolutionary biology as mentioned above in this paragraph.

One of the main variables that define the evolutionary potential of a trait is its heritability. In fact, for evolution to occur the genes determining the expression of a trait need to be passed on to the following generation. The proportion of (additive) genetic variation that is transmitted to the following population with respect to the total phenotypic variation is called (narrow-sense) heritability (Houle 1992; Visscher et al. 2008). Narrow-sense heritability can be estimated from phenotypic data as long as the relevant phenotypic variation and relatedness between individuals is known. The state-of-the-art method to estimate trait heritability are animal models, a special kind of generalized linear mixed models (GLMM) that allow to include the relatedness matrix between individuals as well as other fixed and random effects (de Villemereuil 2018; Kruuk et al. 2008; Wilson et al. 2010). From the estimates of total phenotypic variance and additive genetic variance it is then possible to calculate additional parameters that, coupled with heritability, can help to better understand a trait's evolutionary potential. In fact, heritability estimates alone may be biased by a positive functional and statistical correlation between the amount of additive genetic variance and other variance components such as dominance and epistasis (Hansen et

al. 2011; Houle 1992; Wilson 2008). Evolvability parameters such as the coefficient of additive genetic variation (CV_a) and the mean-standardised additive variance (I_a) overcome these issues by standardizing the additive genetic variance by the trait mean.

Below, I outline some of the processes with the potential to have remarkable effects on parasite ecology and evolution. I first discuss how host choice, at the very beginning of the host-parasite interaction, can affect parasite performance. Then, I focus my attention on sexual selection in parasites. Sexual selection has been shown to be an important evolutionary process in several taxa but its effects on ectoparasites have often been neglected.

Host choice and parasite performance

Host choice is one of the first key phases in the parasite life cycle with key consequences for parasite performance. In fact, parasites acquire their resources from the hosts for their growth, survival, and reproduction (Clayton and Moore 1997). However, different hosts are different environments and as such they can differ in the quality, quantity, and/or easiness of exploitation. Importantly, it has been shown that host variation can affect parasite fitness (Bize et al. 2008; Tschirren et al. 2007). Thus, it can be hypothesized that parasites show some degree of host choice in order to maximize their fitness. For instance, parasites may on the one side prefer hosts in good body condition as they can provide more resources (Christe et al. 2003; Tschirren et al. 2007). On the other side, well-fed hosts may allocate more resources to the immune system and thus be better able to defend themselves from parasitic infestations (Cornet et al. 2014; Krasnov et al. 2005). Thus, the choice between hosts in high or low body condition is likely to have different effects based on the parasite-host system. Additionally, besides their nutritional status, hosts can affect parasite success through inter- and intraspecific differences in anti-parasite behaviour (Barron et al. 2015; Bush and Clayton 2018), morphology (Clayton et al. 2005), and physiology (Christe et al. 2000a; Christe et al. 2007). Hereinafter, I will use

the term "host quality" to refer to the characteristics of the host that increase parasite performance. Despite the limited number of available studies, evidence from wild animal populations suggests intrinsic (e.g. physiological) differences in host quality (Devevey and Brisson 2012; Heylen et al. 2013a). However, these (rare and precious) observations cannot completely eliminate a number of extrinsic confounding factors such as, for instance, spatial parasite heterogeneity or environmental differences in the timing and mode of parasite infestation. In other words, the clustered spatial and temporal distribution observed in many parasite species in the wild cannot be completely ruled out. In order to standardize conditions and rule out (at least part of) this variation experiments carried out in the laboratory would be greatly beneficial.

In ectoparasites, host choice can be affected by several characteristics such as for instance host chemical cues (Dallas and Foré 2013) or morphological characteristics (Caro et al. 2014). The parasite's individual experience (Vantaux et al. 2014) and the abiotic environment such as the season of the year can also play a role (Burkett-Cadena et al. 2012).

Understanding host choice is also relevant to effectively combat disease-transmitting parasites. In fact, disease transmission depends on several parameters that differ between host species and populations such as the host compatibility for a specific pathogen, the vector-host contact rate, vector survival, pathogen transmissibility between host and vector, and so on (see Ostfeld et al. 2010 for a review).

Sexual selection in ectoparasites

More than 150 years ago, sexual selection was proposed to be one of the key drivers of evolution (Darwin 1859; Darwin 1871). Since then, the theoretical framework and empirical evidence on sexual selection has been considerably extended and countless examples of its relevance have been described (Andersson 2019; Jones and Ratterman 2009). In hosts, parasite-mediated sexual selection has

received considerable attention. Parasite can in fact exert multiple selective pressures on host sexual traits and behaviour (David and Heeb 2009; Maan and Seehausen 2011). On the one side, parasites can affect host condition by altering (reducing) the expression of secondary sexual characters (Moller et al. 1999). Moreover, parasites could exploit host sexual signaling to more easily find and exploit the host (Meuche et al. 2016). On the other side, it has been shown that host-parasite coevolution may help to maintain an honest sexual signaling in the host (Maan and Seehausen 2011).

On the contrary, sexual selection in parasites has received little attention so far (Rózsa et al. 2015). In this thesis, I investigate whether parasite traits may be affected by sexual selection. The study of sexual selection in parasites has fundamental relevance for the comprehension of parasite ecology and evolution due to its close relationship with parasite life history, speciation, and host switching. For instance, a recent study showed how two similar populations of feather lice put in hosts of different size developed rapid reproductive isolation following a rapid divergence in body size due to host grooming selection (Villa et al. 2019). Growing evidence suggests that sexual selection indeed occurs in mosquitoes (South and Catteruccia 2016) and lice (Rózsa et al. 2015) while insufficient information is currently available for ticks. Here, I aim at shedding more light on the functioning of sexual selection in ticks.

Ticks as study species

Ticks are arachnids (subclass Acari) belonging to the superorder Parasitiformes, order Ixodida, with three families. Although tick systematics is undergoing considerable reshaping with the rise of molecular techniques, the relationships between the higher level taxa are relatively clear. The three families are the Ixodidae (hard ticks) with approximately 750 species divided in 14 genera, the Argasidae (soft ticks) that include more than 200 species, and the Nuttalliellidae with one single species (Dantas-Torres and Otranto 2020; Guglielmone et al. 2020; Guglielmone et al.

2014). The Ixodidae group is further divided in Prostriate, comprising only the genus *Ixodes*, and Metastriate ticks. Ixodid ticks are obligate but non-permanent hematophagous ectoparasites, and have three stages: larva, nymph, and adult. At each stage they go through a single uninterrupted feeding event (except males of some species that do not feed) and spend most of their life cycle off-host. Feeding typically lasts for 3-10 days and can be divided in two periods: a slow phase during which most of the new endocuticle (inner exoskeleton) is formed (Flynn and Kaufman 2011), followed by a rapid phase (usually the last 24 hours) associated with a strong increase in size before detachment. As hard ticks feed non-stop for several days, they need to firmly attach to the host skin in order not to get easily groomed away. Their feeding apparatus consists of two main parts: a pair of chelicerae and an hypostome (Fig. 2). The chelicerae have the main function of piercing and cutting through the host's skin in order to allow the insertion of the hypostome (Richter et al. 2013; Vancová et al. 2020).

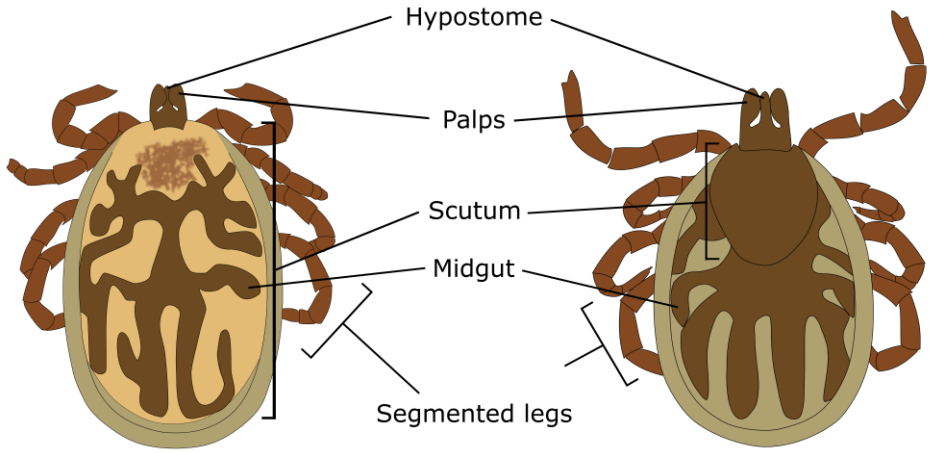


Figure 2. Morphological characteristics of hard ticks (family Ixodidae). Dorsal view of an *I. arboricola* male (left) and female (right).

The latter is equipped with rows of spine-like denticles that physically anchor the tick to the host skin. A cement-like compound further strengthens the attachment by filling the gap between the wounded skin and the tick mouthparts (Suppan et al. 2018).

Adult prostriate ticks can mate both before and after feeding while adult metastriate ticks need a final blood meal for sexual maturation. In all cases, adult females need to feed before egg laying (Sonenshine and Roe 2013). Argasid ticks instead have more complex life cycles and can have multiple short feeding events during every life stage.

Ticks parasitize a wide range of vertebrates including amphibians, reptiles, birds, and mammals. They show substantial variation in ecology and life history which is associated with host specialization (Sonenshine and Roe 2013). Ticks can survive in a wide range of environments from sandy burrows (Hillyard 1996; Nováková et al. 2018) to bamboo bushes (Plantard et al. 2021) and rocky soils (Benoit et al. 2007). Based on their off-host habitat use, ticks that remain in their host's burrow or nest after detachment are called endophilic while those that move freely in the environment are defined as exophilic ticks. In general, ticks can survive temperatures well below 0 °C but are quite sensitive to hot and dry environments (Nelson et al. 2016; Sonenshine and Roe 2013).

Ticks are among the main vectors of diseases of medical and veterinary importance transmitting bacteria (e.g. *Borrelia* spp.), viruses (e.g. tick-borne encephalitis virus), fungi, and protozoans (Boulanger et al. 2019; Dantas-Torres et al. 2012; Hurtado and Giraldo-Ríos 2018; Madison-Antenucci et al. 2020). Indeed, ticks have both direct and indirect negative effects on their hosts whose severity depends on the tick and host species as well as those of any tick-borne pathogens. Direct tick effects are the consequence of blood feeding and include (but are not limited to) anemia, irritation, inflammation, and hypersensitivity putting the host and its immune system under pressure (Heylen and Matthysen 2008; Heylen and Matthysen 2011a; Hurtado and Giraldo-Ríos 2018; Norte et al. 2013; Wall and Shearer 1997). Indirect tick effects on host health are instead linked to tick-borne pathogens. The latter can cause a vast array of lethal and non-lethal diseases such as Lyme disease, tick-borne encephalitis, or ehrlichiosis (Dantas-Torres et al. 2012; Madison-Antenucci et al. 2020; Sonenshine and Roe 2013). Altogether these effects may significantly

reduce host fitness and alter its population dynamics. For instance, tick infestations can lead to nest desertion (Boulinier and Danchin 1996; Burga-Dominguez et al. 2020) and, in some cases, to host death (Gauthier-Clerc et al. 1998; Hoodless et al. 2003). No negative effects have instead been found in other tick-host systems (Hersh et al. 2014), including the one investigated in this dissertation (Heylen and Matthysen 2011a). Understanding the dynamics of tick-borne diseases will not only help to reduce their burden on humans and farm animals but will also help us to predict the effects that are due to the global climate change. It has been predicted that tick distributions may be modified and shift poleward and towards mountainous regions while tick activity may become longer throughout the year, at least regionally (Gilbert 2021; Ogden et al. 2020).

The tree-hole tick Ixodes arboricola

Figure 3. Unfed *Ixodes arboricola* adult female. Photo: G. Fracasso.

In the experimental work that follows I investigate the life history, behaviour and ecology of the tree-hole tick *Ixodes arboricola* Schulze and Schlottke 1929 (Ixodida: Ixodidae). This prostriate hard tick (Fig. 3) is a bird-specialized endophilic tick that can be found in tree holes and nest boxes. Its host range is thus restricted to the bird species visiting these cavities for nesting or roosting, with great and blue tits as its principal host but also including pied flycatchers, blackbirds, treecrepers, and nuthatches (Arthur 1963; Heylen et al. 2014c; Hillyard 1996). The species distribution of *I. arboricola* ranges from Western and Northern Europe to Latvia and the European parts of Russia at East, and Turkey to the South (Keskin et al. 2014; Petney et al. 2012). As all hard ticks (Ixodidae), *I. arboricola* is a non-permanent obligate ectoparasite with a three-stage life cycle: larva, nymph, and adult stage. It feeds once per stage, except for adult males that do not feed. The bloodmeal (engorgement) usually lasts for 3-6 days during which the tick grows in size several

times, and then detaches from the host. Adult males and females are approximately the same size with unfed individuals measuring 2.4-2.7 mm in length while engorged females can instead be up to 6 mm long. The life cycle may be completed in less than a year (Heylen et al. 2014c; Liebisch 1996) although nymphs and adults can respectively survive months and years without feeding (personal observations). Females lay a relatively low number of eggs compared to other Prostriate ticks, most often between 200 and 400 (Van Oosten et al. 2016a; see also chapter III).

Tree-hole ticks are active throughout the year but with life stage differences in seasonal activity. In detail, larvae and nymphs feed on roosting free-flying birds (winter) and on nestlings during the breeding season (spring) while adult females mostly feed on nestlings (Heylen et al. 2014c). Due to its nidicolous habit, *I. arboricola* tends to be vertically transmitted relying on its hosts to spread to other cavities (Van Oosten et al. 2014a). A previous genetic analysis of the *I. arboricola* population in our study area strongly suggests that larvae are the most important stage for tick dispersal (Van Oosten et al. 2014a).

Ixodes arboricola shows specific adaptations associated with its nidicolous ecology. Detachment outside of a cavity would in fact lead to minimal chances of finding a host and survive. Among these adaptations it is worth mentioning that *I. arboricola* mostly detaches during the night when chances that the host is roosting in a cavity are highest. Interestingly, it has been shown that ticks do not detach if the host does not have access to a tree hole or a nest box (Heylen and Matthysen 2010; White et al. 2012). However, the proximate mechanisms that allow the tick to perceive whether the host is in a closed environment remain to be elucidated. After detachment tree-hole ticks move upwards (Heylen 2011; personal observations) in order to moult to the next stage or lay the eggs (Heylen et al. 2014c; Sonenshine and Roe 2013).

Morphologically, sexes can only be distinguished at the adult stage, mainly by the difference in scutum size (smaller in females) as well as hypostome length and shape (Arthur 1963; Heylen et al. 2014a). As adult males do not engorge, their

hypostome only retains the vestigial shape of feeding organ with much less pronounced spine-like denticles (Heylen et al. 2014a; Sonenshine and Roe 2013). Copulation occurs off-host and can take place both before and after adult females have fed. However, engorged females are preferred by males (Liebisch 1996; Van Oosten et al. 2016a). This preference may be due to the fact that unfed adult females still need to find a suitable host where to feed before laying the eggs and may thus be a more risky mating investment for the male. Eggs can be fertilized by several males (multiple paternity) and no sperm precedence (i.e. paternity is equally shared between males) has been identified (Van Oosten et al. 2016a). However, to prevent a reduction in the share of offspring paternity males exhibit mate guarding behaviour. At adulthood, sex ratios are strongly female-biased (Van Oosten et al. 2018). A previous study found no evidence of sex-distorting bacteria and it is currently unclear if the skewed sex ratio is the result of a genetically-induced process during meiosis and/or egg fertilization or the differential survival between sexes throughout the life cycle (Van Oosten et al. 2018).

Ixodes arboricola has been extensively studied both in the wild and in lab conditions by past and current members of the Evolutionary Ecology group (University of Antwerp). Studies spanned a range of research areas and mostly dealt with the tick-host interaction (Heylen and Matthysen 2010; Heylen and Matthysen 2011a; Van Oosten et al. 2016b), ecology and life history (Van Oosten et al. 2014b; Van Oosten et al. 2016a; White et al. 2012). In fact, the abovementioned *I. arboricola* characteristics make this species a good model system respect to other ticks. In this respect, it is worth mentioning that *I. arboricola* can be kept and raised in the lab with individuals surviving for months (larvae, nymphs) or years (adults) without feeding. Moreover, nymphs and adults can be individually marked and feed well on songbirds. The latter can also be kept in captive conditions and easily handled thus offering the opportunity to use marked birds from a population of known history and pedigree. Also, ticks detach in nest boxes maximizing the collection of engorged individuals.

The studies presented in this dissertation have been carried out in a study area composed of a set of woodplots located approximately 10 km south-east of Antwerp (Boshoek), Belgium. The plots have a closed canopy cover dominated by the common oak *Quercus robur* and an understory vegetation mainly composed by bracken *Pteridium aquilinum* and bramble *Rubus fruticosus* (Korsten et al. 2013; Matthysen 2002). The prevalence of *I. arboricola* is generally low and clustered both on hosts and in nest boxes (Van Oosten 2015). However, the removal of the nest material from nest boxes at the end of every breeding season and the occasional collection of wild ticks for experimental purposes may have artificially reduced the natural occurrence of this tick species in our study area. Its main avian host, the great tit, roosts and breeds in high densities in this same area (Heylen et al. 2014c; Matthysen et al. 2001).

The great tit

Figure 4. Drawing of a great tit, Archibald Thorburn (1896). From: Lilford et al. 1885, coloured figures of the birds of the British islands.

The great tit *Parus major* (Fig. 4) is the main host of *I. arboricola* (Hillyard 1996) and was chosen as main study species for this dissertation. By making use of the same host species throughout tick generations and studies I reduced the complexity of the ecological and evolutionary interactions between the tick and its host community. Moreover, the use of a single host species allows to more easily investigate the proximate and ultimate effects of host quality on parasite traits. Also, we can assume that the host intraspecific variation to experimental conditions is lower (or at most equal) respect to the variation between host species thus increasing standardization.

Great tits are passerine birds (Paridae) distributed throughout the Palearctic region (Cramp et al. 1993; Gosler 1993; Lilford et al. 1885). This cavity-nesting songbird mainly inhabits deciduous and mixed forests (Gosler 1993). However, great tits can also be found in small wooded patches such as city parks and gardens (Bańbura and Bańbura 2012). They are 12.5-14 cm long and weigh 13-22 g (Cramp et al. 1993). Their diet is mostly insectivorous, especially during the breeding season and in summer, while in autumn they integrate their food intake with seeds and fruit. Nestlings instead exclusively rely on caterpillars such that the great tit breeding phenology and success heavily depends on the availability and abundance of this food source (Visser et al. 2006). Great tits forage in the lower part of the canopy cover and on the ground where they can get infested by exophilic ticks such as *Ixodes ricinus* and *Ixodes frontalis* (Heylen et al. 2013a; Heylen and Matthysen 2010; Špitalská et al. 2011). Great tits mainly use nest boxes for roosting in winter (November-February) and for breeding in spring (March-June). During the roosting period cavities are occupied by a single bird that can here get infested by *I. arboricola*. In our study population, great tits have been found infested by both *I. arboricola*, *I. ricinus*, and *I. frontalis* (Heylen et al. 2014c).

Great tits establish breeding territories in early spring (February-March), generally in the same area where females roosted during winter. They show high site fidelity irrespective of their mate survival, provided that the previous breeding event was successful (Harvey et al. 1979). Great tits are monogamous birds although a small percentage of extra-pair paternity has been detected (Brun et al. 1999). They build a new nest made mainly with moss at every breeding season. After laying 5-11 eggs females start the incubation phase that lasts for 12-15 days. Nestlings become homeothermic around day 8 after hatching and fledge when they are 17-21 days old (Gosler 1993).

The ubiquitous distribution of great tits associated to their resilience to stress manipulation made this songbird a model species in ecology (Marcel et al. 2003; Senar et al. 2017), evolution (Charmantier et al. 2017; Laine et al. 2016; Senécal et al.

2021), and behaviour (Hardman and Dalesman 2018). Great tits have also been studied in the context of tick-bird interactions (Heylen 2011; Kocianová et al. 2017; Van Oosten 2015). In particular, the Evolutionary Ecology group (University of Antwerp) has more than a decade-long tradition in the study of great tit-tick interactions.

Besides tree-hole ticks, great tits can host several other parasites and pathogens (Dufva 1996) that can interact with each other in complex ways in the host (Hellard et al. 2015). The bird population used in this dissertation was occasionally infested by fleas, with nest boxes hosting a rich community of arthropods (Baardsen et al. 2021). To the best of my knowledge, interactions between ectoparasites in great tits are not known. As regards pathogens, tree-hole ticks have been shown to carry *Rickettsia* sp. and *Borrelia burgdorferi* s.l. bacteria but there is currently no evidence that these pathogens can be transmitted from *I. arboricola* to the host (Heylen et al. 2013b; Špitalská et al. 2011). However, it cannot be excluded that other unidentified pathogens are transmitted to great tits (Baardsen et al. 2021; Hellard et al. 2015).

In our study area, great tits mostly breed and roost in nest boxes whose density is approximately of 10 nest boxes/ha (Matthysen et al. 2001). The resident great tit population is part of a long-term monitoring study in which all breeding pairs and nestlings occupying a nest box are individually identified by means of a metal ring and followed throughout their life cycle (Matthysen 2002). Thus, the majority of the breeders have known pedigree, i.e. the identity of their parents is known (Korsten et al. 2013). When needed for experimental reasons birds are caught through mist-netting or at the nest boxes. All captures have been carried out by qualified personnel under licence of the Flemish Ministry (Agentschap Natuur en Bos) and approval by the Ethical Committee of the University of Antwerp, Belgium (in compliance with the Directive 2010/63/EU).

Outline of the dissertation

In this dissertation I investigate the evolutionary ecology of a hematophagous ectoparasite, the tree-hole tick *I. arboricola* feeding on its main songbird host, the great tit *Parus major*. Songbirds can be easily kept and handled allowing to carry out standardized infestations and to recover the ticks after detachment (see **chapters II**, **III**, and **IV**). This allows to individually follow and measure both the host and the parasite multiple times throughout their life cycle thus allowing to disentangle the direct and indirect selective pressures that parasites and hosts have on each other. To the best of my knowledge this opportunity has mostly been neglected up to date. I studied the individual variation and evolutionary potential of tick traits to comprehend how natural and sexual selection shape variation in the parasite traits, its performance and life-history strategies. In order to do so, I devoted myself to study ticks at the individual level by making use of the same methods and statistical techniques that are usually reserved to vertebrate species (e.g. animal models). I present four studies, one for each chapter, that aim at shedding more light on several aspects of parasite individual variation and on the evolutionary potential of parasite traits. An additional chapter organically synthesizes the findings previously presented and suggests promising future avenues of research.

In **Chapter II**, I report a study in which I investigated on-host micro-habitat preference in ixodid ticks feeding on birds. Anecdotical and field data suggest that ixodid ticks are only found on the head of avian hosts. However, it is unclear if this observed pattern is the result of tick attachment preferences, namely parasite micro-habitat selection while on host, or rather the outcome of differences in host grooming efficiency between body areas, i.e. host anti-parasite behaviour. Understanding the mechanisms of on-host habitat selection is a crucial step for the comprehension of host-parasite interactions and coevolution since it can affect parasite feeding success and survival as well as parasite load on the host. Additionally, it has been shown that the transmission of tick-borne pathogens is enhanced by the close proximity between ticks (Ogden et al. 1997; Randolph 2011;

Voordouw 2015). Thus, a better comprehension of tick distribution on the host body would also provide further insights on how co-feeding can affect the spread of vector-borne diseases in the wild. Interspecific and interstage variation in tick attachment sites as well as tick preference were studied. Findings are discussed in the light of the proximate and evolutionary causes that may drive the observed pattern. Additionally, this study was a prerequisite to optimize the infestation procedure that would later be used in **chapter III** and **IV**.

In **Chapter III**, I describe a study on the individual variation of some fundamental *I. arboricola* life-history traits, their relationship with tick performance, the phenotypic and genetic correlations between these traits, and their evolutionary potential. I measured the tick life-history traits associated with feeding and fitness – namely feeding time, engorgement weight, moulting time, and number of hatched eggs – as well as their associated success parameters – namely feeding, moulting, egg-laying success, and survival. I then set up animal models using the relatedness between tick individuals (pedigree) to estimate trait heritability and evolvability (i.e. CV_a: coefficient of additive genetic variation, CV_r: coefficient of residual variation, I_a: mean-standardised additive variance). I proceed discussing the ecological and evolutionary significance of these findings and point out the great and underexploited potential of individual-based experiments in the study of parasitology and host-parasite interactions.

In **Chapter IV**, I report the effects that individual hosts have on tick feeding parameters and fitness. While a considerable number of studies has been carried out on how parasites affect hosts, much less attention has been paid to the effects that individual host characteristics have on their parasites. Although some studies strongly suggest that host quality, as perceived from the parasite perspective, differ between individual hosts in the wild (Devevey and Brisson 2012; Heylen et al. 2013a) investigations are still scant and lack standardization. Also, it is currently unclear if these host effects on parasites are heritable, thus potentially exerting long-lasting selective pressures on parasites. To shed some light on the topic, I studied variation

in great tit quality (from the tree-hole tick perspective) by measuring the correlation in tick performance between different tick stages (larvae and nymphs) feeding on the same birds. To remove the extrinsic environmental variation typical of wild conditions I investigated host quality in the laboratory. The bird relatedness (pedigree) was used to estimate heritability of host quality by means of animal models. Finally, I investigated if variation in tick performance could be explained by bird sex, age, body condition as well as its change during the experiment, and haematocrit. Tick performance measures included the on-host (attachment success, feeding time, engorgement weight and feeding success) and off-host phase (moulting time, moulting success and overall survival).

Methodologically, to carry out the studies presented in **chapter III** and **IV** a founder population of tree-hole ticks was caught from the wild and raised in laboratory for two complete generations. Larvae and nymphs were fed on wild-caught great tits while adult females were fed on great tit nestlings in the wild accordingly to the natural attachment preference of *I. arboricola* (Heylen et al. 2014c). Both the ticks and the birds were individually followed throughout the study (Fig. 5). To follow ticks individually across three different feeding events and two ecdyses, every individual was singularly kept in a univocally labelled glass vial since larva engorgement. Nymphs and adult females were marked by clipping part of one limb that was then completely regenerated after moulting (see supplementary information **chapter III** for further details). The relatedness matrix between ticks and between most of the infested birds was known. As birds were caught from the wild (adult birds) or infested at the nest (nestlings) this experimental provided semi-natural conditions. In this way, I managed to reduce the drawbacks of model systems exclusively kept and studied in captivity or in lab conditions (Poulin and Keeney 2008; Schmid-Hempel and Ebert 2003).

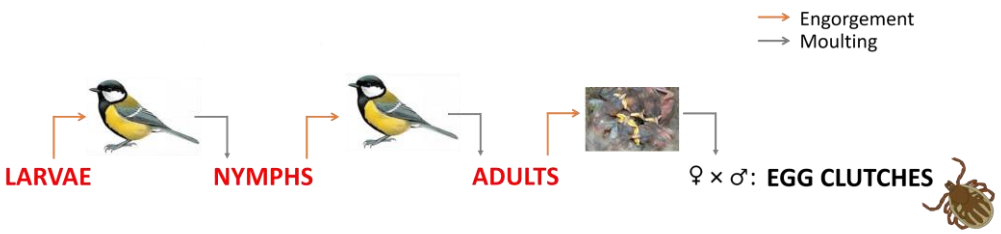


Figure 5. Overview of the experimental study setup used for chapter III and IV. Two consecutive tick generations were investigated.

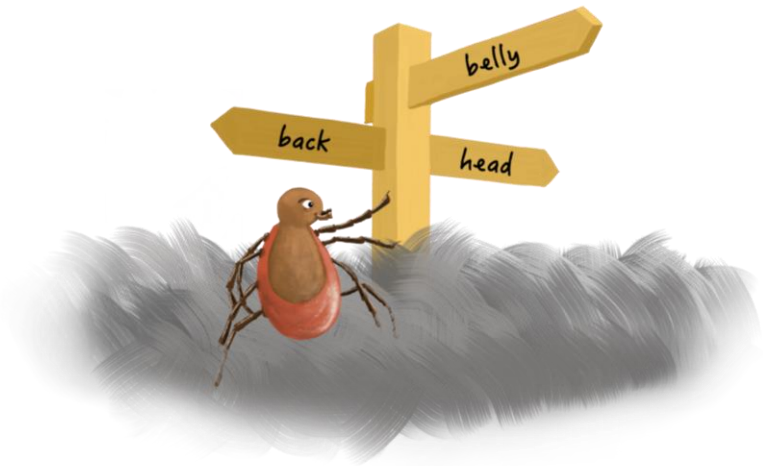
In **Chapter V**, I show my results on the individual variation in *I. arboricola* adult female attractiveness, namely male mate choice. Sexual selection is one of the most important drivers of evolution shaping the morphology, behaviour, and life history of species (Andersson 2019). Despite the available literature on the mating behaviour and physiology of ixodid ticks (Kiszewski et al. 2001; Sonenshine and Roe 2013), the importance of sexual selection in shaping tick biology is not fully understood. Hence, I further investigated male mate choice in *I. arboricola* as this species exhibit peculiar reproductive characteristics. In detail, in *I. arboricola* heavier engorged adult females produce more offspring as is the case for many other tick species (Chen et al. 2009; Ginsberg et al. 2016; Gray 1981; Ma et al. 2013; Van Oosten et al. 2016a). Hence, males mating with them should benefit from a higher fitness. Moreover, *I. arboricola* ecology and behaviour show several characteristics that favour the evolution of male mate choice (Barry and Kokko 2010; Bonduriansky 2001; Edward and Chapman 2011). I used two different experimental setups that allowed to discriminate if ticks used olfactory or tactile and visual cues during mate choice. Results point out our insufficient understanding of tick mating strategies and sensory capabilities.

Finally, **Chapter VI** presents a synthesis of all previous findings and provides a comprehensive discussion of the possible proximate and ultimate processes that could explain the findings shown in this dissertation. By taking into account parasite ecology and evolution I give a general overview of the individual variation and evolutionary potential of *I. arboricola* and generalize these outcomes to other hematophagous ectoparasites. I conclude pointing out the overlooked potential of an

individual-based approach for the study of ectoparasites and suggest promising new research avenues.

Chapter II

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



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Abstract

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

Introduction

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

Ectoparasites are a particularly interesting group in this respect because they can potentially feed on many different locations on the host, yet often their distribution on the body surface is rather narrow. Ticks are no exception as they are generally only found on specific parts of the host's body (Balashov 1972) with attachment sites differing greatly between host, parasite species and life stage. Among blood-sucking ectoparasites ticks are of great veterinary and medical importance as they transmit a vast array of pathogens such as bacteria, protozoans, viruses and fungi (Goodman et al. 2005; Sonenshine et al. 2002; Sonenshine and Roe 2013). Extant ticks are divided in two major clades, Argasidae (soft ticks) and Ixodidae (hard ticks), with Nuttalliellidae as an additional monotypic group. Argasid

(Argasidae) and ixodid (Ixodidae) ticks have different feeding characteristics with the Ixodidae spending more time attached to the host skin and feeding only once for each instar (Binnington and Kemp 1980; Uspensky 2008). In this study we focus on the distribution of ixodid ticks on birds.

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

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

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

Tick species (n° ticks)	Host family (n° infested birds)	Attachment site	Region (country)	Reference
Ixodes ricinus (218)	Phasianidae (>18[a])	Head	Scotland, South England (UK)	Elston et al., 2001; Hoodless et al., 2003
Ixodes ricinus (2493)	Turdidae (446)	Head	Six federal states (Germany), Burgundy (France)	Gregoire et al., 2002; Klaus et al. 2016
	Muscicapidae (91)			
	Sylviidae (88)			
	Paridae (41)			
	Acrocephalidae (66)			
Ixodes spp. likely *I. ricinus* (1588)	Prunellidae (29)			
	Fringillidae (36)			
	Phylloscopidae (15)			
	Troglodytidae (12)			
	Motacillidae (10)			
Amblyomma aureolatum (699)	Parulidae (7)	Head and throat	Paraná (Brazil)	Arzua et al., 2003
	Conopophagidae (1)			
	Furnariidae (10)			
	Thraupidae (7)			
Ixodes auritulus (18)	Thamnophilidae (5)			
	Troglodytidae (7)			
	Turdidae (104)			
	Passerellidae (1)			

Ixodes brunneus (na)	Bombycillidae (3) Fringillidae (29) Turdidae (2) Corvidae (1) Icteridae (2) Passerellidae (3) Columbidae (1)	Head, neck	Georgia, Arkansas, Tennessee, Virginia, North Carolina (USA)	Luttrell et al., 1996
Haemaphysalis leporispalustris (1171) *Ixodes scapularis* (13)	Passerellidae (157) Turdidae (204) Parulidae (29) Icteridae (2) Corvidae (2) Certhiidae (1) Mimidae (1) Fringillidae (2) Cardinalidae (1) Troglodytidae (1)	Head	Wisconsin (USA)	Nicholls and Callister, 1996

Amblyomma nodosum (17) *Amblyomma calcaratum* (11) *Amblyomma longirostre* (22) *Amblyomma maculatum/ triste* (2) *Haemaphysalis leporispalustris* (1) *Haemaphysalis juxtakochi* (38)	Turdidae (12) Cardinalidae (8) Parulidae (9) Vireonidae (2) Icteridae (1) Tyrannidae (1) Passerellidae (2)	Head (78%), cloaca (10%), rest of the body (12%)	Louisiana (USA)	Mukherjee et al., 2014
Ixodes lividus (40)	Hirundinidae (27)	Head	Lower Saxony, North Rhine-Westphalia (Germany)	Walter et al., 1979; Hudde and Walter, 1988
Ixodes arboricola (819)	Paridae (98) Sittidae (1) Muscicapidae (1) Passeridae (2)			
Likely *Ixodes hirsti* (na)	Meliphagidae (na)	Head	South Australia	Kleindorfer et al., 2006
Ixodes spp. (116)	Laridae (3) Spheniscidae (2)	Head, neck, chest (to a lower extent)	New Zealand	Heath, 2006

Ixodes uriae (~7012)	Alcidae (88)	Foot webs, back, breast, belly, crissum, tail (Alcidae)	Alaska (USA), British Columbia (Canada)	Choe and Kim, 1987, 1988; Morbey, 1996
	Laridae (28)	Wings (50%), other body parts (50%, Laridae)		
	Phalacrocoracidae (4)	Wings (35%[b]), back (30%[b], Phalacrocoracidae)		
Ixodes signatus (224)		Head (73%[b], Alcidae)		
		Head (60%[b], Laridae)		
		Head (58%[b], Phalacrocoracidae)		
Ixodes uriae (296)	Laridae (~195)	Thighs, legs, foot webs, belly, cloaca, wings, head and neck	Scotland, England (UK)	Danchin, 1992; Barton et al., 1995
Ixodes uriae (~ 10800)	Spheniscidae (3)	Head (adults, nymphs); lower body parts (larvae)	Crozet archipelago (French Southern and Antarctic lands)	Gauthier-Clerc et al., 1998

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

An obvious hypothesis to explain the observed pattern of tick attachment in birds is that ticks aggregate on the head because there they are least vulnerable to grooming. Grooming is one of the most important defences against ectoparasites (Bush and Clayton 2018; Clayton et al. 2010) with evidence of selective pressures acting both on hosts and parasites (Clayton et al. 2015). Nevertheless, we are not

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

Materials and methods

Study system

For this study we used the three ixodid tick species that are the most common on European songbirds. These three species strongly differ in habitat requirements, host specificity, and phenology (Heylen et al. 2014a). The tree-hole tick *Ixodes arboricola* Schulze and Schlottke 1929 is an endophilic bird-specialised hard tick. Its entire life cycle takes place in cavities, and it relies on its host to spread to other tree holes and nests (Van Oosten et al. 2014a). The main hosts of *I. arboricola* are shared with *I. ricinus* (Linnaeus 1758), an exophilic ground-dwelling tick. The latter can be found on an extensive range of vertebrates though adults mainly parasitize large

mammals (Comstedt et al. 2006; Humair et al. 1993; Olsén et al. 1995). *Ixodes ricinus* transmits many pathogens such as bacteria (e.g. *Borrelia burgdorferi* sensu lato, *Rickettsia* spp.), viruses (e.g. tick-borne encephalitis virus) and protozoans (e.g. *Babesia* spp., *Trypanosoma* spp.) (Sonenshine and Roe 2013). Finally, *Ixodes frontalis* (Panzer 1798) is a scarcely known species that is considered to be bird specific, and it has been recovered from many bird species including great tits *Parus major* Linnaeus 1758 (Arthur 1952; Hillyard 1996; Tsapko 2017). It can be found on the understory vegetation where it shares the habitat with *I. ricinus* (Heylen et al. 2014a).

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

Experiments were performed between 2012 and 2019. The number of experimental birds and the number of ticks administered varied between experiments according to trapping success of birds, availability of cages and number of ticks available for infestation. Great tits were captured from the wild within 25 km from the city centre of Antwerp (Belgium), and kept individually in cages (80 × 40 × 40 cm). Food and water were provided *ad libitum*. Canaries were selected from a laboratory-based population kept indoor in single-sex aviaries at a room temperature of 19 − 24 °C and artificial light. Before infestation each bird was given at least 48 hours to acclimatise. In total, 66 great tits (39 males and 27 females; 5 birds used

twice) were infested with larvae or nymphs of *I. arboricola*, *I. frontalis* or *I. ricinus*. Twelve canaries (4 males and 8 females) were infested with *I. arboricola* nymphs.

Ixodes arboricola ticks came from a laboratory colony established in 2008 with the addition of wild individuals in 2017. They were fed on great and blue tits *Cyanistes caeruleus* (for further details see Heylen and Matthysen 2010). Larvae of *I. frontalis* were obtained from one engorged female while *I. ricinus* nymphs were the second generation from adults collected near Berlin in 2014 and bred on Gerbils *Meriones unguiculatus* (company: INSECT SERVICES GmbH, Berlin, Germany). All ticks were kept in similar abiotic conditions (relative humidity > 84%; temperature range 15 − 20 °C). *Ixodes arboricola* was kept in the dark, while *I. frontalis* and *I. ricinus* were kept under an 18:6 light:dark cycle.

Tick exposure procedure

Nymphs were placed on the bird's skin using tweezers, while, because of their small body size, larvae were transferred from the vials to the skin using a paintbrush. We successfully used this technique previously (Heylen et al. 2017; Heylen and Matthysen 2010; Heylen et al. 2014b). The head part was defined as the area on top of the body between the beak and the ears (included) while back and belly were respectively the dorsal and ventral areas delimited by the junction between humerus and scapula on one side and the tail on the other side. Immediately after exposure, every bird was put into one or more air-permeable cotton bags, depending on the type of experiment (see below). The outer bag (10 × 20 cm) was tightly closed such that the ticks could not escape. After 90 minutes, the bird was gently removed from the bag and released into its cage. Cotton bags were inspected and any ticks found in a bag were counted and killed in ethanol (80%). In virtually all cases, the ticks were not damaged. The body part of tick attachment was checked by inspecting birds for feeding ticks 60 − 72 hours after tick exposure. We thoroughly screened the birds' skin by blowing and brushing the feathers apart with tweezers (Heylen et al. 2009; Heylen et al. 2014c). We classified ticks in four categories (Fig. 1). Ticks placed on the

bird '*Ta*' were either found inside a cotton bag '*Tb*', or assumed to be infesting the bird '*Ti*'. At inspection 60 – 72 hours later ticks on the bird (Ti) were further subdivided in ticks engorging '*Te*' and separately counted for each body part (*Te'*) or missing '*Tm*'. Following ratios were calculated per bird individual for each experimental condition and tick × life stage combination: ticks on the bird after 90 minutes (Ti/Ta), ticks not infesting the bird after 90 minutes (Tb/Ta), ticks missing (likely due to grooming) after 60–72 hours (Tm/Ti), overall infestation success (Te/Ta), and body part preference (Te'/Te).

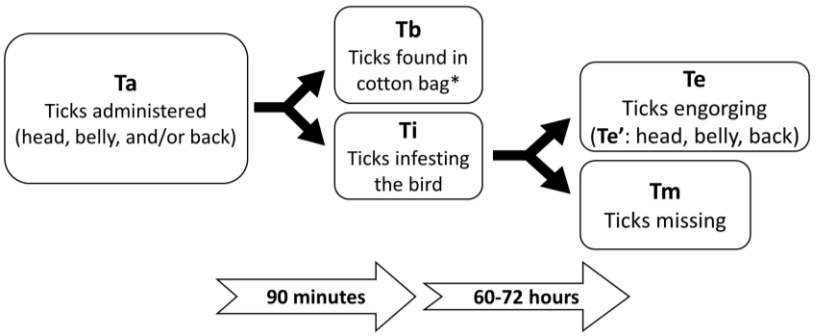

Figure 1. Overview of the experimental workflow and of the variables considered. After 90 min, ticks placed on birds (Ta) are assigned to one of the following categories: Tb, ticks found inside a cotton bag; or Ti, ticks infesting the bird's body (i.e. Ta-Tb), and thus potentially attached and feeding.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

Experiment 1: tick exposure on great tits without grooming restrictions

This experiment was carried out to investigate the abovementioned ratios when hosts were not prevented from grooming and tick movements on the host body were not constrained. The final tick attachment success (Te/Ta) and tick distribution on the bird's body is therefore the result of tick movement, attachment preference and host behaviour. Tick exposure success after 90 minutes (Ti/Ta) needs to be interpreted in the presence of host grooming efforts. Fifteen great tits received approximately 120 *I. arboricola* larvae each, which were placed on one of the three body parts: head, belly or back (5 birds in each treatment). Twenty-eight birds

received 15 *I. arboricola* nymphs each (20 birds were infested on the head, 4 on the belly, 4 on the back) (Table 2 for an overview).

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

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

Exp 4	*I. arboricola* nymphs	N° canaries	2	3	3	**8**	
	Ticks/bird = 12; Total = 96	N° ticks recovered	43 (179)	0	0	**43**	**(45)**
		Total birds		**2**	**29**	**78**[c]	
		Total ticks recovered	**2012 (155)**	**4**	**2**	**2018**	**(62)**

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

Experiment 2: tick exposure on great tits with grooming restrictions

This experiment tested if ticks differ in attachment success when applied to different body parts while preventing the host from grooming. Each bird individual received ticks on the head, belly or back depending on the treatment. After placing the ticks on the host body, the bird was immobilized to avoid preening with the beak or grooming with claws and beak. Specifically, we inserted the bird in a small cotton bag that perfectly fitted the body, and which was subsequently clamped with a plastic barrette close to the neck such that the ticks that were placed on back or belly were partially hindered to move towards the head and *vice versa*. The head was kept outside the small bag. Legs were also placed outside this small bag, through small holes. Both legs were held together with a cotton elastic hair band. The bird was then put (horizontally) into a larger outer cotton bag that was tightly closed. After 90 minutes the two bags were removed and inspected. This setup made it possible to separately count ticks infesting each body part in the absence of grooming (Ti/Ta) after 90 minutes. As the bags were removed after 90 minutes, '*Ti*' ticks were subsequently free to move, and resulting parts of attachment (60 − 72 hours later) can differ from the parts where the ticks have been placed initially.

For practical reasons related to the number of birds and ticks available, we performed slightly different variations of the experiment for different tick species. Twelve birds were infested with 15 *I. ricinus* nymphs each on one body part only (6 birds on the head, 3 on the belly, and 3 on the back). Similarly, 3 birds were infested

with approximately 80 *I. frontalis* larvae (one bird for each treatment: head, belly, and back). For *I. arboricola*, 12 great tits were infested with 30 nymphs each divided over three body parts: 10 on the head, 10 on the belly, and 10 on the back (Table 2).

Experiments 3 and 4

We performed two additional small-scale experiments. In Experiment 3 we placed in total approximately 120 *I. frontalis* larvae (40 on the head, 40 on the belly, and 40 on the back) on a single great tit whose grooming was restricted (see description experiment 2). In experiment 4 we used canaries to test the tick attachment success on a novel songbird species. Twelve canaries were infested with 12 *I. arboricola* nymphs each. Four canaries − exposed without grooming restrictions (see description experiment 1) − were infested on the head while eight canaries − exposed with grooming restrictions − were infested with ticks on head (N = 2), belly (N = 3), or back (N = 3).

Statistical analysis

Results were analysed using Generalized Linear Models (GLM) with binomial distribution (logit link). The proportion of ticks that did not infest the experimentally exposed body part after 90 minutes (Tb) and the proportion of ticks feeding 60 − 72 hours after exposure (Te) were set as dependent variables in the models. These will be referred to as, respectively, failure to attach (Tb/Ta) and attachment success (Te/Ta) (note that these do not sum to one, because of ticks that were missing). Treatment (head, belly, back), tick species and life stage were set as independent categorical variables. The latter two factors were grouped in one variable called "tick batch" (AL: *I. arboricola* larvae, AN: *I. arboricola* nymphs, RN: *I. ricinus* nymphs, FL: *I. frontalis* larvae). Separate models for each experiment were run to analyse the two dependent variables as defined above. Differences in attachment success between birds exposed to *I. frontalis* larvae were not statistically analysed due to the very small sample size. Tick batch was not included in the model for experiment 2 since

birds infested with *I. arboricola* and *I. ricinus* nymphs were analysed separately. The proportion of *I. arboricola* nymphs that failed to attach in experiment 2 (i.e. Tb/Ta) was analysed through a Chi-squared test. To test if the attachment success to a specific body part differed with respect to the exposed body part, we ran a GLM model for each experiment with treatment as independent categorical variable and the proportion of attachment to the head as dependent variable. The effect of tick life stage on attachment success to the head was investigated by setting treatment, batch, and their interaction as fixed effects. Two outliers were removed from experiment 1 in all relevant models since the larvae recovered exceeded the number of larvae approximately put on the bird. Likelihood ratio tests (LRT) were used to obtain the Chi square values of the models. We used the "multcomp" package in R to calculate the pairwise comparisons (Hothorn et al. 2008). P values less than 0.05 were considered significant. Except where indicated, percentages are given with respect to the total number of ticks put on the birds for the same treatment and batch. Data analysis was performed in R v 3.5.2 (R Core Team 2020 2018).

Results

Experiment 1: tick exposure without grooming restrictions

In total, 1306 *I. arboricola* larvae (Te, out of Ta = 1800) attached to the birds with 1305 larvae found on the birds' head (72.5% of all larvae. Table 2) and 1 on the birds' belly. The attachment success to the head significantly differed between ticks exposed to the belly and back: 394 out of 600 (66%) attached to the head when put on the head while 369 out of 600 (62%), and 542 out of 600 (90%) attached to the head when put on the belly and back respectively. Seventy-one larvae (3.9% of all larvae) were found in the cotton bag after exposure (Tb). Similarly, all 277 *I. arboricola* nymphs (Ta = 420) feeding on great tits attached to the head independently of the part of exposure (Table 2). The attachment success to the head did not differ significantly between exposed body parts: 201 out of 300 nymphs (67%) attached when put on the head while 42 out of 60 ticks (70%), and 34 out of 60

ticks (57%) when put on the belly and back respectively. Forty-two nymphs failed to attach (Tb), of which 18 when placed on the head (6%), 9 (15%) on the belly, and 15 (25%) on the back (overview of mean nr. of 'Tb' ticks in Table 3).

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

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

[a]Four canaries without grooming restriction.

The proportion of ticks that successfully attached on the body part where the original exposure took place, strongly differed among the treatment groups (χ_2^2 = 1235.12, *P* < 0.001; N = 41). Eventually, all attached nymphs were found on the head, no matter the body part where they were released. The GLM model on the proportion of ticks found in the bag after the exposure showed a significant interaction between treatment and tick batch (i.e. tick species and life stage) (χ_2^2 = 10.734, *P* = 0.005; N = 41). This result is mainly due to a higher proportion of nymphs found in the bag (Tb) when exposed to the back respect to larvae exposed to the same body part. The attachment success to the head differed significantly in respect to treatment (χ_2^2 = 6.978, *P* = 0.031; N = 41) with larvae initially put on the belly having a significantly lower attachment success compared to larvae put on the back (P = 0.025). Tick life stage had no effect on attachment success to the head (χ_1^2 = 0.007, *P* = 0.933; N = 41).

Experiment 2: tick exposure with grooming restrictions

In the birds infested with *I. ricinus*, 81 nymphs (Ta = 180) attached in total: 76 nymphs on the birds' head (42% of all nymphs. Table 2), 3 (7%) on the birds' belly and 2 (4%) on the back. Specifically, 70 out of 90 nymphs (78%) attached to the head when put on the head, while only 5 out of 45 (11%), and 1 out of 45 (2%) attached to the head when put on the belly and back respectively. Three ticks (3%) failed to attach when put on the head, 24 (53%) on the belly, and 31 (69%) on the back. The proportion of ticks that attached to the exposed body part was significantly different between treatments (χ_2^2 = 75.7, *P* < 0.001; N = 12). Moreover, the proportion of ticks that failed to attach was significantly higher when ticks were put on the back or on the belly compared to ticks put on the head (both pairwise comparisons: *P* < 0.001). For *I. ricinus* the attachment success to the head differed significantly with respect to treatment (χ_2^2 = 108.83, *P* < 0.001; N = 12) with ticks initially put on the head having a significantly higher attachment success (both comparisons P < 0.001).

As regards *I. arboricola*, all 152 feeding nymphs (Ta = 360) were attached to the head (42% of all nymphs and 127% of nymphs put on the head, see Table 2) while no nymph attached to back or belly. Ninety-five nymphs (40%) failed to attach when placed on the birds' back or belly but only 14 (6%) when placed on the head. The proportion of ticks that failed to attach was significantly different between exposed body parts ($\chi_1^2 = 28.226$, $P < 0.001$; N = 12). In the three birds infested with *I. frontalis* all 63 engorging larvae (Ta = 240; 26% of all larvae) were feeding on the head while no tick attached to the back or belly. Specifically, 50 out of 80 (63%) attached to the head when put on the head while 1 out of 80 (1%) and 12 out of 80 (15%) attached to the head when put on the belly and back respectively. Moreover, 137 out of 160 (86%) *I. frontalis* larvae failed to attach when administered on the belly or back part. In contrast, no larvae were found in the bag (Tb) in the great tit infested on the head.

Experiments 3 and 4

As regards the single bird exposed to larvae of *I. frontalis* without grooming restrictions, we found 63 larvae (53% of all larvae. Table 2) feeding on the bird, all of which attached to the head. Similarly, in the 4 canaries infested on the head without grooming restrictions 33 ticks (Ta = 48; 69%) attached to the head while no tick attached to back or belly. Only 7 ticks (15%) failed to attach after exposure (Tb). When canaries were prevented from grooming, 43 ticks (Ta = 96) attached to the 8 birds, again all of them on the head (Table 2): 18 out of 24 nymphs (75%) attached when put on the head while 6 out of 36 (17%), and 19 out of 36 (53%) attached to the head when put on belly or back respectively. The proportion of ticks found in the bag after exposure differed between treatments (χ_2^2 = 20.611, $P < 0.001$; N = 8): 4 ticks (17%) failed to attach when put on the head compared to 23 ticks (64%) and 7 ticks (19%) for the belly and back treatment respectively. The attachment success to the head differed significantly with respect to treatment (χ_2^2 = 22.813, $P < 0.001$; N =

8) with ticks initially put on the belly having a significantly lower attachment success when compared to ticks put on the head or back (both comparisons P < 0.006).

Combined data from all experiments

Considering all ticks put on all experimental birds we found a total of 2018 ticks (Ta = 3264) feeding 60 − 72 hours after exposure: 2012 ticks attached to the head, 4 ticks attached to the belly and 2 ticks to the back (Table 2, Fig. 2. Ticks attached to the head for every bird: Supplementary Table S1). Within the head, most of the ticks were located next to the eyes and in the area between the beak and the eyes. The GLM model run on the data from all tick batches − excluding *I. frontalis* due to the very small sample size and the birds infested on multiple body parts − showed a significant effect of treatment (χ_2^2 = 1416.640, P < 0.001, N = 65) on the proportion of ticks found attached to the same part where they were initially exposed. The proportion of ticks that did not infest the bird after 90 minutes (Tb/Ta) was significantly related to the interaction between treatment and experiment (χ_2^2 = 9.370, P < 0.01, N = 65): infestation failure was lowest for the head treatment in all experiments but the proportion of ticks that failed to attach and the treatment associated with highest attachment failure differed between batches and experiments. Specifically, ticks in exp. 2 exposed to the back and ticks in exp. 2 and 4 exposed to the belly showed a much higher infestation failure compared to the other experiments. Considering all

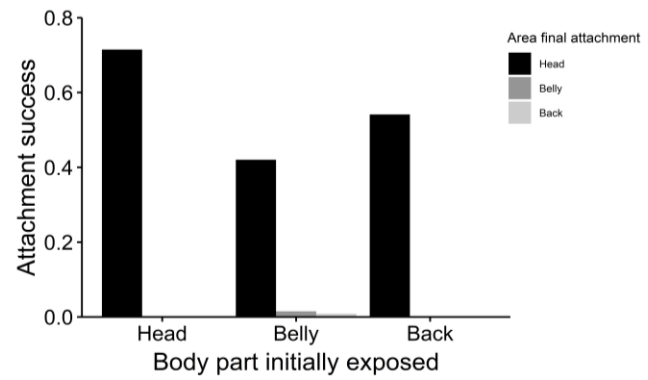

Figure 2. Proportion of attached ticks with respect to body part where attached and body part where initially exposed. Data from all experiments were pooled, weighed based on the number of infested birds in each treatment, and averaged. The single great tit infested with *Ixodes frontalis* and the birds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

experiments together, we found a lower infestation failure for ticks put on the head (Fig. 3).

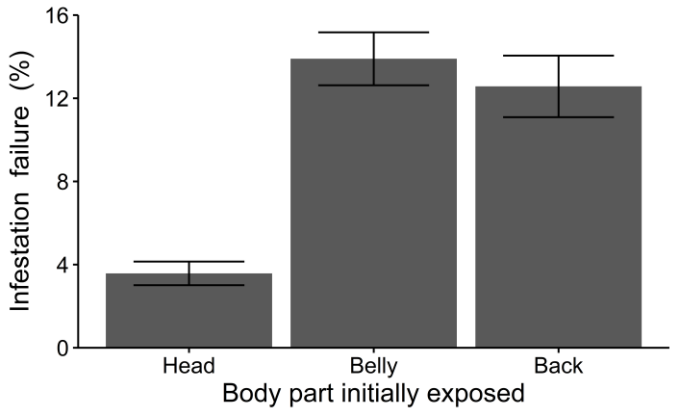

Figure 3. Percentage of ticks found in the bag after 90 min of exposure. Data include average percentage of *Ixodes arboricola* larvae and nymphs (experiments 1 and 4), and *Ixodes ricinus* nymphs (experiment 2) that failed to attach with respect to the body part of exposure. Error bars represent ± 1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

Overall, 25% (807 out of 3264) of the ticks put on the birds was never found (Tm). A number of factors hindering tick detection can be pointed out. The small size of unfed ticks (especially larvae) and the ticks' habit to crawl at the base of the plumage are likely the most important ones. Feathers needed to be shifted to locate the feeding ticks making a very accurate screening sometimes too stressful for the bird. Alternatively, ticks may have remained on the bird without attaching during the tick exposure procedure and have been groomed away by the host during the following hours. When attachment success on the head was compared between exposed body parts considering experiment as fixed effect (exp. 1, 2, 4), we found a significant interaction between experiment and treatment (χ^2_2 = 44.043, P < 0.001; N = 61). In particular, exposure to the head led to the highest attachment rate in all experiments but experiment 2 and 4 showed a lower attachment success on belly and back compared to experiment 1. When all these three experiments were pooled together the attachment success to the head differed significantly with respect to treatment (χ^2_2 = 19.830, P < 0.001; N = 61). Ticks initially put on the head showed a significantly higher attachment success only when compared to ticks put on the belly (P < 0.001, Fig. 4).

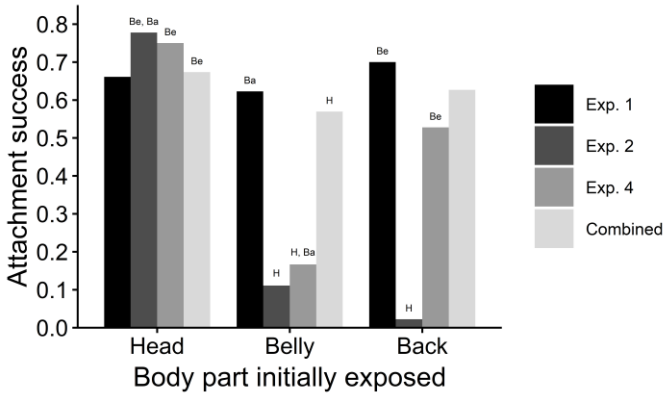

Figure 4. Proportion of ticks attached to the head with respect to the body part initially exposed for all main experiments (exp. 1, 2, 4). Letters above bars indicate a significant difference ($P < 0.05$) with respect to head (H), belly (Be), or back (Ba) of the same group.

Discussion

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

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

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

Tick attachment preference can be adaptive for a number of non-mutually exclusive reasons. Primarily, preference for the head could have evolved in response to host grooming that causes severe fitness reduction in the parasite (Clayton et al. 2005; Waite et al. 2012). Ticks that are groomed away, are generally critically injured and even if they survive may have little opportunity for re-infestation. Generally, birds are very effective in reducing ectoparasites, whereby the beak – that has access to the complete body except for the head – plays a very important role. It has been shown that minor beak deformities increase ectoparasite load (Clayton et al. 2010; Cotgreave and Clayton 1994) and that parasites act as a selective pressure on beak shape (Villa et al. 2018). Since most songbirds do not show allo-preening, the head is a safe place for the tick to attach as it cannot be groomed via the beak, and it is also

difficult to be reached by claws. Hence, we believe that preening avoidance is the first proximate causation for the pattern observed. Interestingly, more scattered infestation patterns have been found in *I. uriae*, a generalist hard tick feeding on seabirds (Arthur 1963; Dietrich et al. 2014). *Ixodes uriae* can be found attached to different body parts such as the lower body parts depending on the host species and tick life stage (Table 1). Seabirds differ from passerine species in several aspects of ecology and morphology which may explain the difference in the pattern of tick attachment. In addition, the different shape of the beak and the webbed claws may result in a reduced grooming efficiency thus reducing the selective pressure acting on attachment sites. Unfortunately, to the best of our knowledge no data on grooming efficiency in marine birds is available to investigate this hypothesis. Also, several aspects in the life history of *I. uriae* differ from other ixodid ticks (McCoy and Tirard 2002).

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

Experiments with different ixodid species show that when ticks engorge in parts with scarce blood supply, feeding takes slightly longer and leads to a reduced weight of engorgement (Balashov 1972). Compared to other body parts, the birds' head is a well vascularised area covered by a thin skin layer (Pass 1995; Stettenheim 2000). This may facilitate tick attachment and a fast and effective engorgement. In particular, the orbit, the oral cavity and the nasal cavity are areas of thermal

exchange supplied by a main source of blood splitting in numerous blood vessels (Porter and Witmer 2016). Also, a higher feather density might protect ticks from the airflow while the bird is in flight (Choe and Kim 1991). Hence, choosing the head might reduce feeding time (and its associated risks), protect against host grooming and directly increase tick fitness. Tick fecundity is in fact correlated to weight after engorgement (Gray 1981).

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

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

In conclusion, our results show that different species of ixodid ticks have a consistent preference for attaching on the head of their songbird hosts and move to

the head even if placed on other body parts. This experimental finding is consistent with literature reports on several tick species on songbirds and other terrestrial birds. Hence, we suggest the existence of a consistent pattern of attachment across ixodid species with the exception of *I. uriae* on seabirds. Similar patterns of attachment might also occur in different host taxa and have an impact on tick survival and evolution as well as on disease transmission. We hope future studies will help addressing this question.

Chapter III

Predictors of individual performance and evolutionary potential of life-history traits in a hematophagous ectoparasite

Fracasso G, Heylen D, Van Dongen S, Elst J, Matthysen E.

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Abstract

Little is known about the intraspecific variation of parasite life-history traits and on how this variation may affect parasite fitness and evolution. We investigated how life-history traits predict success of individual tree-hole ticks *Ixodes arboricola* and estimated their evolutionary potential, as well as genetic correlations within stages and phenotypic correlations within and across stages. Ticks were followed individually over two generations while allowed to feed on great tits *Parus major*. After accounting for host and tick maternal effects, we found that short feeding times and high engorgement weights strongly increased moulting success. Moulting time was also positively correlated with feeding success in adults. In larvae and nymphs we found negative phenotypic correlations between engorgement weight and both feeding and moulting time, the latter supported by a negative genetic correlation. We found sex-related differences in feeding time (longer in male nymphs) and moulting time (longer in male larvae but shorter in male nymphs). Also, time since the last feeding event (set experimentally) reduced larval and nymphal fitness while it increased adult female fitness. Furthermore, we found significant heritability and evolvability, i.e. the potential to respond to selection, for engorgement weight and moulting time across all stages but no significant heritability for feeding time. Our findings suggest that variation in tick fitness is shaped by consistent individual differences in tick quality, for which engorgement weight is a good proxy, rather than by life-history trade-offs.

Introduction

Host-parasite interactions are among the most dynamic co-evolutionary processes, as host and parasite exert mutual selective pressures ultimately leading to the emergence of adaptations and counter-adaptations (Carius et al. 2007; Clayton and Moore 1997; Poulin 2007; Sorci et al. 1997). Comprehending how parasite life-history traits covary, their relative contribution to parasite fitness and their evolutionary potential will show us how parasites may adapt to new selection pressures and evolve different life-history strategies (Barrett et al. 2008; Clayton et al. 2015; Clayton and Moore 1997), providing a crucial basis for the development of predictive tools for disease monitoring and prevention (Anderson et al. 2010; Dantas-Torres et al. 2012; Dronamraju 2004; Levin et al. 1999; Tolle 2009).

Evolution of parasite traits will only occur if selection affects heritable components of phenotypic variation but can be constrained by trade-offs among traits both within and across life stages (Aguirre et al. 2014; Kruuk et al. 2001; Morrissey et al. 2012; Teplitsky et al. 2014). Therefore, to understand and ultimately predict evolution, it is crucial to estimate the selection pressures as well as the additive genetic variation and genetic and phenotypic covariances between traits. In order to optimize their fitness, parasites are expected to evolve traits that facilitate host exploitation and transmission without necessarily leading to higher virulence, i.e. damage to the host (Alizon et al. 2009; Clayton and Tompkins 1994; Heylen and Matthysen 2011a; Poulin 2007). Hosts in turn can be expected to evolve either resistance traits preventing or limiting the exploitation by parasites, or tolerance traits that alleviate the fitness consequences of the infection/infestation (Mazé-Guilmo et al. 2014; Poulin 2007).

So far, very few studies on parasites have focused on individual-level variation in traits and fitness, as most research focuses on factors such as prevalence, transmission and virulence at the population level. While many studies have examined the heritability and (micro)evolution of host traits related to resistance or tolerance (Ayres et al. 2015; Boulinier et al. 1997; Hill 1998; Kause et al. 2012; Mazé-

Guilmo et al. 2014; Williamson and Kumar 2006), very few studies have quantified genetic variation for parasite life-history traits. Even in parasites with great economical and public health relevance such as hard ticks (Ixodidae), the genetic underpinning of phenotypic traits has hardly been investigated (but see Li et al. 2005; Lysyk 2010; Madder et al. 1996). A major factor contributing to this general knowledge gap is the difficulty of tracking individual parasites through their life cycle, as they are small, reproduce in large numbers, often have low survivability, and may go through multiple stages. In addition, life cycle switches between on-host and off-host habitats complicate the rearing and tracking of parasites and imply very different selective environments that need to be studied separately (Poulin 2007; Poulin and Keeney 2008; Van Oosten et al. 2014b).

Ectoparasites are a taxon where these methodological challenges can be overcome, since most of them can be readily observed and tracked, and spend the entire life cycle on or near the host (Clayton et al. 2015). Nevertheless, few experimental studies were carried out on ectoparasite evolutionary potential. For instance, Bush et al. (2019) showed that host behaviour can select for adaptive responses in feather lice in just a few dozen generations, and even may lead to reproductive isolation (Villa et al. 2019), but these studies did not track individual parasites. Some individual-based studies estimated heritability of a foraging phenotype in mites (Durkin and Luong 2018; Durkin and Luong 2019; Jia et al. 2002; Nachappa et al. 2010) and infectious behaviour of ticks (Fragoso-Sanchez et al. 2011; Li et al. 2005; Lysyk 2010; Madder et al. 1996; Young et al. 1995) but we are not aware of any studies on individual traits and how they correlate with fitness. Obligate but non-permanent ectoparasites such as mites and ticks provide an additional experimental advantage as they can be allowed to feed on hosts but can be monitored off-host for survival, development (e.g. moulting) and reproduction (Van Oosten et al. 2018; Van Oosten et al. 2016a).

Ticks are common hematophagous ectoparasites of terrestrial vertebrates, and after mosquitoes are considered the second main vector of diseases for humans and

farm animals (Dantas-Torres et al. 2012; Parola and Raoult 2001). Hard ticks feed only once per life stage and spend the rest of their lives off-host. During their life cycle they experience a series of major challenges potentially generating selective pressures on life-history traits. First, ticks have to find and reach a suitable host in often complex and vast environments (Carr and Salgado 2019; McMeniman et al. 2014; Tomás and Soler 2016). Second, as observed in other ectoparasites, they need to overcome host behavioural (Clayton et al. 2010) and physiological defences (Owen et al. 2010) before and during attachment and feeding (Fracasso et al. 2019). Thus, optimal feeding time may be determined by a trade-off between the amount of blood ingested and exposure to host defences (Bize et al. 2008; Reid et al. 2014). Third, off-host ectoparasites have to survive and moult while coping with adverse environmental conditions and predation (Leal et al. 2020). Lastly, a mating partner has to be found and eggs laid in a suitable environment.

With the exception of the well-established positive correlation between adult female engorgement weight and clutch size (Chen et al. 2009; Ginsberg et al. 2016; Gray 1981; Ma et al. 2013; Van Oosten et al. 2016a), the fundamental relationships between phenotypic traits, individual fitness and heritability have hardly been investigated in ticks. Most quantitative genetic research has rather focused on tick control and disease prevention, such as heritability of pesticide resistance (Fragoso-Sanchez et al. 2011; Li et al. 2005), susceptibility to infection (Young et al. 1995), or heritability to cause paralysis (Lysyk 2010). Only a single study reported a small heritable component for adult body weight in *Rhipicephalus appendiculatus* under laboratory conditions (Madder et al. 1996).

To investigate individual variability in life-history traits and their evolutionary potential, we used a songbird-tick system, namely the tree-hole tick (*Ixodes arboricola*) and its major host, the great tit (Parus major; Heylen 2011; Van Oosten 2015). The advantage of this host-specialized tick is that the same host species can be used for all stages thus reducing both practical and conceptual complexity. We monitored individual ticks throughout their life cycle and over two generations. Our

approach can be split in four parts: first, we measured trait variability and investigated which traits predicted individual fitness components such as attachment success, feeding success, moulting success, overall survival, and egg-laying success. Second, we measured phenotypic correlations within and across stages and genetic correlations within stages to investigate the potential trade-offs between traits during the entire tick life cycle. Third, we evaluated trait evolutionary potential by quantifying trait heritability, namely the proportion of phenotypic variation due to heritable genetic variation in a population, as well as evolvability, namely a population's ability to respond to selection through adaptive genetic variation (Hansen et al. 2011; Houle 1992). Fourth, we estimated the variance in phenotypic traits associated with maternal effects (tick clutch), and the environmental effects, mainly embodied by the individual host. We did all this for each life stage separately, thus allowing for a comparison on the relative importance of genetic, clutch and environmental effects across stages.

Materials and methods

Study species

Tree-hole ticks (*Ixodes arboricola*, Schulze & Schlottke 1929) were experimentally reared and fed on great tits (*Parus major*, Linnaeus 1758), in our study region probably its main host species (Heylen et al. 2014c; Van Oosten et al. 2014b). This tick is an obligate nest-dwelling ectoparasite feeding on birds roosting or breeding in natural cavities (Heylen et al. 2014c; Van Oosten et al. 2014b; White et al. 2012). As most ixodid ticks, *I. arboricola* feeds once as larva and nymph before moulting, with adult females, but not adult males, feeding a third time before egg laying (Sonenshine and Roe 2013).

Great tits are small passerine birds (16 – 21 g) inhabiting woodlands, parks and gardens throughout Europe, part of Asia and North Africa (Cramp et al. 1993). All birds came from a free-living population in the Boshoek area (51° 7' 59" N, 4° 31' 1" E) near Antwerp, Belgium (Korsten et al. 2013; Matthysen 2002). Here, great tits use

nest boxes for breeding (April – June) and for roosting (October – February). Great tits in this population are regularly infested with *I. arboricola* (Arthur 1963; Heylen et al. 2014c; Literak et al. 2007) as well as *Ixodes ricinus* (Heylen et al. 2013a; Hubalek et al. 1996) and more rarely with *Ixodes frontalis* (Heylen et al. 2014b; Heylen et al. 2013b).

General study design, trait and success variables

Between 2017 and 2019, two consecutive tick generations were raised in semi-natural conditions starting from wild-caught adult ticks (F0, see Fig. 1 for the study design). Larvae and nymphs were fed on wild-caught great tits held captive for the duration of the infestation (10 days), while adult females were fed on nestling great tits in nest boxes in the field. We thus mimicked the natural feeding strategy of *I. arboricola* whereby adults mostly feed on nestlings while immature stages can feed on both adults and nestlings (Heylen et al. 2014c). In this way, we made optimal use of the limited temporal availability of wild great tit nestlings. Nymphs and adults were marked individually while unfed larvae were only identified at the clutch level (protocol details below).

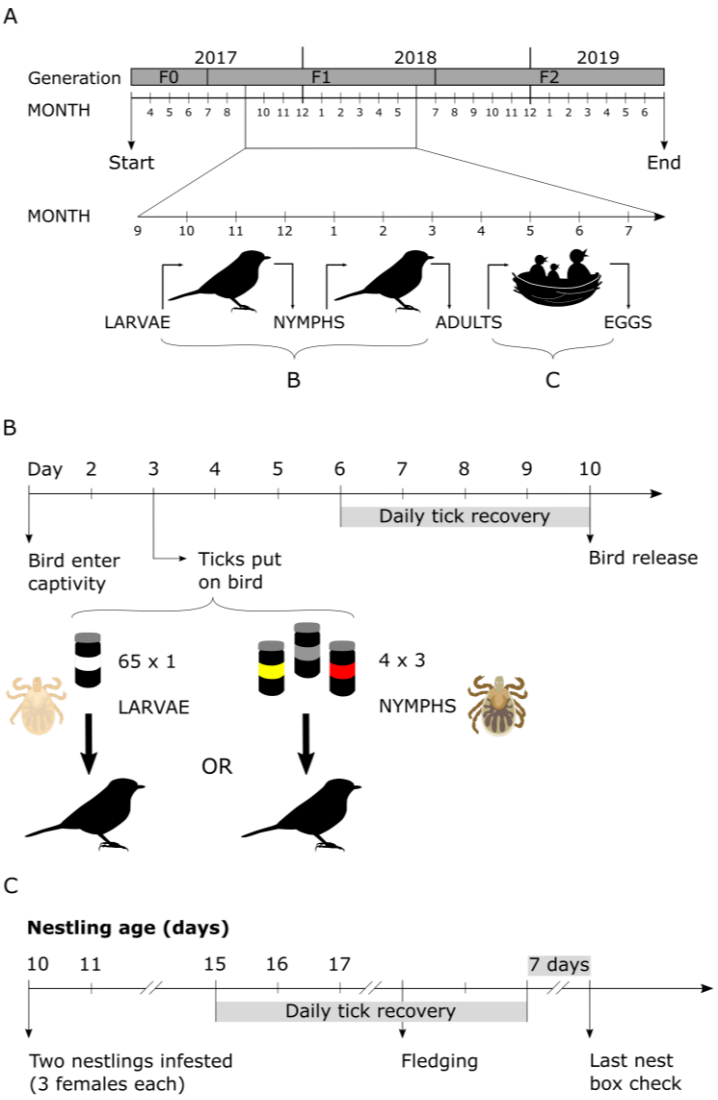

Figure 1. Study design. A) Overview across years. Adult ticks were collected in the wild (F0) and bred for two generations (F1, F2) starting in March 2017. Within each generation, larvae and nymphs were fed on adult great tits between October and March, and on nestlings during the breeding season. B) Overview of infestation procedure for ticks put on adult great tits in lab conditions. After two days of acclimatization every bird was infested with 65 larvae from one clutch or 12 nymphs from 3 clutches (4 from each clutch). Starting from the third day of infestation engorged ticks were collected daily. C) Infestation procedure for adult ticks. Three ticks were put on each of two 10- or 11-day old nestlings in the same nest. Collection of engorged ticks started 5 days later and continued daily until all ticks were recovered or until 2 days after fledging. If at this date ticks were still missing, a final inspection was carried out 1 week later. See main text for further details.

We quantified three traits for each life stage and four traits per generation. These were 1) feeding time (all stages), 2) engorgement weight (all stages), 3) moulting time (larvae and nymphs), and 4) number of hatched eggs (adult females). Feeding time may reflect a trade-off between the amount of resources taken from the host and exposure to host defence (e.g. grooming and immune defence). As mentioned already, engorgement weight is known to be positively correlated with fecundity and subsequent survival. For instance, acquired host resistance leads to lower survival and/or engorgement weight, possibly mediated by shorter feeding durations (Gebbia et al. 1995; Jones and Nuttall 1990). However, the relationship between engorgement weight and survival when hosts have no acquired resistance remains unknown. We hypothesize that tick performance will be positively correlated with engorgement weight, a proxy for the amount of resources available. Moulting time affects how rapidly a tick can feed, and thus influences generation time. As *I. arboricola* hosts occupy tree cavities for a short period during the breeding season and winter, ticks that moult quickly can feed a second time within the period of host availability and gain a fitness advantage (Heylen et al. 2014c; Heylen et al. 2012). However, ticks with higher engorgement weights may also need a longer moulting time due to the higher amount of blood ingested. Hence, there may be a trade-off between engorgement weight (resources acquired) and the advantage of a short moulting time, especially for immature stages. Lastly, the number of hatched eggs is a key measure of female reproductive investment. These traits will be linked to the success variables in further analyses (see below).

Feeding time was calculated as the time elapsed between day of infestation and day of collecting the tick. Engorgement weight was individually measured twice to the nearest 10^{-2} mg, and the average used for analyses. We defined moulting time (called "premoulting period" in some studies) as the number of days elapsed between collection and emergence from exuvia (former exoskeleton). The number of hatched eggs was defined as the number of larvae emerging from the clutch and counted in the vial where eggs were laid. This method provided a proxy of female

fitness without interfering with egg integrity, and takes into account egg viability. The number of hatched eggs was not counted for F0 for practical reasons (storage in semi-transparent vials hindering larval counts) while for the other generations it was approximated to the nearest five.

The following success variables were measured, which chronologically reflect the crucial events in the tick life cycle, starting from attachment on the host: attachment success, feeding success, moulting success, survival, and egg-laying success. As we aimed to study all variables at the individual level, success traits could only take binary values (yes/no). We did not include egg production success (i.e. number of eggs produced), as it overlapped with the trait "number of hatched eggs". We defined attachment success (adult females only) when an infested tick was not found in the bag 1 h after infestation (see below). Feeding success (nymphs and adult females) was defined as the recovery of an engorged tick. Engorged ticks are easily distinguished from unfed ones (change in shape and size). We did not recover any living ticks that had not engorged (see also supplementary information, SI hereinafter). Moulting success (larvae and nymphs) was defined as the successful moulting once a tick was engorged. Survival success describes the survival of an infested tick until successful moulting (larvae and nymphs) or egg hatching (adult females) thus encompassing feeding and moulting success. Finally, egg-laying success (adult females) was defined as laying at least one egg (see Table 1 for an overview of the variables).

Table 1. Definitions of the main variables in this study and life stage to which they apply: larvae (L), nymphs (N), adult females (F).

Variable	Description	Life
Tick traits		
Feeding time	Days elapsed between infestation and recovery.	L, N, F
Engorgement weight	Weight after engorgement in 10^{-2} mg.	L, N, F
Moulting time	Days between recovery and completion of moulting.	L, N
Hatched eggs	Number of larvae hatched from a clutch.	F

Tick success parameters (all yes/no)		
Attachment success	Tick not found in the bag 1 h after infestation.	F
Feeding success	Infested nymph or female recovered engorged.	N, F
Moulting success	Engorged larva or nymph that completed ecdysis.	L, N
Survival success	Infested nymph or female that completed ecdysis.	N, F
Egg-laying success	Engorged adult female that laid at least one egg.	F
Covariates and random effects		
Sex	Tick sex, assessed at the adult life stage.	L, N, F
Year	Calendar year of infestation.	L, N, F
Infestation attempt	Tick attached at the first or second infestation[a].	F
Fasting time	Days between recovery or hatching and next infestation.	L, N, F
Batch	Period of simultaneous infestation of a group of birds.	L, N
Feeding event	ID of infestation (bird × batch; nest ID for adult females).	L, N, F

[a]Within stage

F0 Generation

In winter 2016, 58 adult male and 54 adult female ticks were collected from four wooded areas within 25 km from Antwerp. Most ticks were collected several months before the nestling season and stored in single-sex vials thus limiting the chances of paternity from wild adult males. We gathered ticks from multiple locations to boost genetic variation in our founder (F0) population, since earlier studies showed moderate genetic differentiation among these populations (Van Oosten et al. 2014a). Adult females were put on 10- or 11-day old nestlings (16 nests, one tick per nestling) for feeding. Nest boxes were checked daily for engorged ticks starting four days after infestation. All *I. arboricola* stages exhibit negative geotropism (Heylen and Matthysen 2010) and can thus be collected on the nest box lid with minimal nest disturbance. If not all ticks were recovered, the nest box was checked one last time nine days after fledging. No ticks were found in the nest material when a subset of these nests (N = 9) was inspected thoroughly. Following

engorgement, the F0 females were allowed to mate with two randomly chosen males, to ensure a maximal set of fertilized clutches. Since *I. arboricola* may mate prior to feeding and multiple paternity is common (Van Oosten et al. 2016a), the number of fathers per clutch could have been one, two or even more in case of pre-engorgement mating.

F1 and F2 larvae and nymphs

Each year, larvae (October – December) and nymphs (January – March) were fed on adult great tits individually held in indoor cages (80 × 40 × 40 cm) for 10 days. Each cage was equipped with a nest box for bird roosting, thus promoting tick detachment (White et al. 2012). Since we could house no more than 24 birds simultaneously, a cohort of larvae or nymphs was typically split into two or three infestation sessions (separated by one or more weeks), henceforth "batches". As some birds were used in more than one batch, we will henceforth refer to ticks feeding on the same bird in the same batch as a "feeding event". We provided standardised artificial daylight (10 h 30 min including dawn and sunset, independently from season), temperature (20 °C), and relative humidity (55 ± 3%). Cages were surrounded by an 11-cm wide trench of water to prevent ticks from escaping.

Every bird was given 48 h to acclimatise before infestation. We infested birds with randomly chosen ticks from clutches that contained sufficient individuals for infestation, thereby aiming to maximize the number of clutches represented in every batch. Ticks were put on the bird head following earlier procedures (Heylen et al. 2017; Heylen and Matthysen 2010; Heylen et al. 2014b) and in accordance with natural attachment behaviour (Fracasso et al. 2019). Every bird was infested with either approximately 65 larvae, or 12 nymphs with four nymphs from each of three different clutches. Since larvae were too small to be marked, each bird received larvae from a single clutch. In each year, some clutches were used to infest more than one bird with larvae, in successive batches, including clutches with a low

recovery success in the initial batch. Nymphs were marked individually by clipping part of one limb with a scalpel in humid conditions, within 2 h before infestation (for details see SI). Trials carried out by the authors before the beginning of the study showed no substantial differences in behaviour or performance between clipped and unclipped ticks (results not shown). To prevent impairment in host finding behaviour, the first pair of limbs holding the Haller`s organ was not clipped (Romanenko et al. 2016; Sonenshine and Roe 2013). Immediately after infestation, birds were put in an air-permeable cotton bag for 1 h (Fracasso et al. 2019; Heylen et al. 2017; Heylen et al. 2014b). Then, every bird was released in its cage, and any unattached ticks found in the bag were counted. Ticks almost exclusively detached in nest boxes; we fully inspected them starting from the third day after infestation. Nest boxes were checked every day for five days, and engorged ticks were collected (Fig. 1*B*). The time window of tick collection matched the normal detachment period of *I. arboricola* (White et al. 2012). During this period, all birds spent the night in the nest box. A soaked sponge was put at the bottom of the nest box to increase humidity and promote tick survival. At the end of every batch birds were inspected. Any ticks still attached were collected and assigned an additional day in feeding time since they could have detached at the earliest by the next morning due to the natural detachment behaviour of *I. arboricola* (Heylen and Matthysen 2010). These ticks represented less than 7% of all detached ticks (5% larvae and 13% nymphs).

F1 and F2 adult females

During the breeding season, adult female ticks (individually marked as for nymphs) were placed on 10- and 11-day old great tit nestlings. After inspecting and weighing all nestlings, two of them with approximately average weight and similar development were infested with 3 ticks each, preferentially originating from different clutches. Nestlings were then put individually in a small air-permeable cotton bag for 1 h inside the nest box (Heylen and Matthysen 2011b). Unattached ticks were collected from the bag and re-used a second time for another nest. In this case, the

life-history traits were based on the second infestation, but success parameters were based on the first infestation. Hence, unattached adult females after the first infestation were considered as unsuccessful regardless of the outcome of the second infestation. Since very few F0 females were recovered at four days, nest boxes for F1 and F2 were checked daily from the fifth day until recovery of all engorged females, or until two days after fledging (Fig. 1*C*). As for adult F0 females, the nest box was checked one last time nine days after fledging if not all ticks had previously been recovered. Females recovered after day eight were assigned an unknown feeding time since they likely had detached earlier but remained temporarily hidden in the nest material thus potentially increasing measurement error. After engorgement, F1 and F2 adult females were mated with one randomly chosen non-sibling male.

Rearing conditions and monitoring

In between feeding events, all ticks (except unfed larvae) were individually kept in glass vials in darkness at 20 °C and 85% relative humidity. Each engorged nymph and adult female was rinsed, weighed and stored within 24 h from collection; with a few exceptions larvae were rinsed and weighed within eight days (maximum 11 days) after collection. Ticks were rinsed for 1 min with a solution of distilled water and sodium hypochlorite (0.005%) to remove dirt particles and reduce the risk of fungal infections. Larval weight did not change with time until weighing (linear model estimate = 0.006, P = 0.89). Ticks were checked daily for occurrence of moulting, egg laying, or egg hatching except for a few occasions where checks occurred with two- or three-day intervals.

We started the F1 generation with larvae from 51 clutches (F0), and the F2 generation with 48 clutches. Per generation, we obtained 1600 to 1800 engorged larvae, approximately 330 engorged nymphs, and about 60 engorged adult females. The number of birds infested per tick stage and generation varied between 59 and 92 (Table S1 in SI). The models predicting attachment success, feeding success, and survival included 1349 and 304 unfed nymphs and adult females, respectively.

Analyses of moulting success, genetic and phenotypic trait correlations, and Animal Models were based on engorged ticks: 3462 larvae, 661 nymphs, and 182 adult females. The sample size reduction from unfed to engorged ticks is in line with the feeding success observed in the wild (authors' pers. obs.).

Statistical analyses

Predictors of fitness

Predictors of attachment, feeding, moulting, survival, and egg-laying success were analysed at the individual tick level by fitting separate Bayesian generalized linear mixed models with a Bernoulli distribution using the "brms" (v. 2.15.0) package (Bürkner 2017; Bürkner 2018) in R 4.0.5. For attachment, feeding, and survival success, we set feeding time and engorgement weight in the previous life stage, as well as moulting time into the present stage, as predictors. For the models on moulting success and egg-laying success, we fitted feeding time and engorgement weight in the same stage, adding infestation attempt (i.e. whether the adult female attached at the first or second infestation) for egg-laying. Except for egg-laying success, we included fasting time (number of days elapsed between recovery or hatching and the next infestation) as a covariate, since this time was set by the experimenter and not by the ticks. Year was also included as covariate. In the model on egg-laying success, moulting time and fasting time were excluded as they were unknown for F0 females. Batch, feeding event, tick clutch, and nest identity (adult females only) were specified as random effects. In models on adult females, batch was not specified as nestlings were infested over a short period of two to three weeks. With the exception of year, fixed effects were mean-centered and standardized to a variance of one. Four parallel chains were run with default weakly informative priors and model convergence was checked. In the result section, we only report effects whose 95% credible intervals do not overlap zero (see SI for all models and results). For clarity, 95% credible intervals will be abbreviated as "95% CI" while 95% confidence intervals (see below) will be written in full.

Sources of genetic and environmental variation

Variation in tick traits was analysed by fitting a tri-variate Animal Model (generalized linear mixed model) in a Bayesian framework (Brommer et al. 2019; de Villemereuil 2019; Kruuk et al. 2008; Wilson et al. 2010) using the "MCMCglmm" (v. 2.32) package (Hadfield 2010; Hadfield 2019). Flat and weakly informative extended priors were chosen (see SI). A different model was run for each life stage: larvae, nymphs, and adult females.

The response variables (traits) in the models were engorgement weight, feeding time, moulting time (larvae and nymphs) and number of hatched eggs (adult females). Since moulting time and feeding time were right-skewed, they were normalized by raising them to the negative exponent maximizing the Shapiro-Wilk test score (*W*). All traits were then mean-centered and scaled to a variance of one. Tick pedigree (relationship matrix), clutch, feeding event, and batch were included as random effects, except for adult females where we included year as fixed effect instead of batch. As F1 and F2 adult females were kept singly, immediately collected after engorgement, and only allowed to mate with a single male of known identity, the paternal and maternal link of most of the clutches could be attributed exactly. In the two cases where paternity from wild adult males could not be excluded, we assigned the clutch to an unknown father. With regard to the F0 pedigree, we attributed a different dummy father for every female that laid eggs. Since we had no prior information on F0 male characteristics, heritability estimates should be only weakly affected by multiple paternity, if it occurred. In the latter case, estimates may be slightly underestimated. Feeding event includes the effects of the tick environment while feeding, namely the effect of host individual quality (heritable and non-heritable components, such as behavioural and physiological defences) at the time of infestation. In addition, feeding event includes the effect of tick feeding density (i.e. variation in the number of ticks that attached) and any other minor sources of variation during captivity and infestation. Feeding event thus explains the remaining variance at individual bird level after batch-level effects (experienced by all

birds simultaneously) are accounted for. Additional sources of variation at the tick level are clutch of origin, sex, and fasting time (see above). As the contribution of tick clutch is estimated independently of the parental genetic contribution, it can be considered a maternal effect. Sex was included as fixed effect for larvae and nymphs. However, as it could only be determined in the adult stage, ticks that never reached maturity were considered of unknown sex. Thus, we executed an analysis with, and without this subset of non-surviving ticks. In adult females, we also included nestling identity as random effect so that the variance explained by feeding event in larvae and nymphs was here split in two parts: nestling identity accounting for characteristics of the individual host, and nest identity for the environmental conditions shared by nestlings.

Covariance between different groups of random effects was fixed to zero (e.g. between clutch and batch) since estimating these covariances was outside the aims of the study; this also reduced model complexity while likely having negligible effects on the partitioning of variance. For each model five chains were run in parallel. Convergence within and between chains was checked in the "coda" (v. 0.19.3) and "MCMCvis" (v. 0.13.5) packages (Plummer et al. 2006; Youngflesh 2018) following de Villemereuil (2012). Means and 95% CI of the posterior distributions were calculated on the third chain of each model. As heritability is bounded between zero and one, lower 95% CI very close to zero do not provide clear evidence of substantial heritability. In such cases, heritability estimates were considered consistently different from zero when the shape of their posterior distributions was approximately gaussian. See SI for all models and results.

Evolutionary potential

For every trait we estimated the additive genetic variance (V_a), as well as the variance attributed to batch, feeding event and clutch. Heritability was calculated as V_a over the total phenotypic variance (V_p), while taking into account the fixed effects abovementioned. Similarly, we calculated the ratio of V_p explained by each random

effect (main text) as well as the total variance explained by every effect (SI). As heritability can be a misleading proxy for the evolutionary potential of a trait (Hansen et al. 2011; Houle 1992; Wilson 2008), we also calculated the coefficient of additive genetic variation (CV_a), the coefficient of residual variation (CV_r), and the mean-standardised additive variance (I_a) following Houle (1992). Since trait values were transformed and mean-centered, we could not directly calculate CV_a, CV_r, and I_a using V_a from the Animal Models because trait means become zero and variances are estimated on a different scale (Garcia-Gonzalez et al. 2012; Houle et al. 2011). Hence, we fitted an additional model with scaled, mean-centered but untransformed traits whose results were comparable to those of the transformed model. We did this for nymphs, but not for larvae and adult females because of model instability when using untransformed traits. Then, additive genetic variances (V_a) and trait means obtained from the latter model were back transformed to the observed data scale using the function "QGmvparams" (customized model definition accounting for fixed effects) in the "QGglmm" (v. 0.7.4) package (de Villemereuil 2020; de Villemereuil et al. 2016) and used to calculate heritability on the observed scale (h_o^2), CV_a, CV_r, and I_a (see also SI).

Genetic and phenotypic correlations

We estimated genetic correlations between traits (and 95% CI) within but not between stages, as the Animal Models were single stage. Phenotypic correlations were estimated from standardised and normalized data using Kendall's tau correlation with 95% confidence intervals (10^4 bootstrap iterations) using the "NSM3" (v. 1.16) package (Schneider et al. 2018). Correlations were considered significant when 95% credible or confidence intervals did not overlap zero.

Results

General trait information

Besides the expected marked differences in engorgement weight, feeding and moulting time also significantly increased between developmental stages (Table S2). In particular, feeding time increased from 4.5 to 4.8 days between larvae and nymphs and to 5.6 days in adult females (Wilcoxon rank sum test: $P < 0.001$ for all comparisons) while moulting time increased from 20 to 23.9 days between larvae and nymphs (Wilcoxon rank sum test: $P < 0.001$; Table S2, Fig. S1). The sex ratio of freshly moulted adults was 1:2.46 (M:F, N = 654). Feeding success was about 70% for larvae and 57% for nymphs with the latter having a higher moulting success: 92% nymphs and 75% larvae. All 182 engorged adult females were alive at mating, but 22 never oviposited. The average number of hatched eggs was 168 (211 when excluding females that never oviposited, range 0 – 445). Eight clutches never hatched (see also Table S1, S2).

Predictors of fitness

Higher attachment success (adult females) was correlated with longer moulting time to the adult stage (Table 2, S3; Fig. 2 for a graphic overview of outcomes), but not with engorgement weight or feeding time in the previous stage. Interestingly, adult female feeding success also increased with moulting time while no such association was found in nymphs. Moulting success increased with engorgement weight and decreased with feeding time in both larvae and nymphs, whereby engorgement weight had a stronger effect in larvae and feeding time in nymphs (Table 2). No trait had a significant effect on overall survival in either nymphs or adult females. Lastly, egg-laying success was not related to any measured trait but was lower for ticks that attached at the second infestation attempt (Table S7). Interestingly, fasting time negatively affected both feeding success (Fig. S2) and survival in nymphs, and moulting success in larvae, while it positively affected feeding success and survival in adult females. Among the random effects, batch

explained a higher proportion of the variance compared to clutch and feeding event in the feeding success and overall survival of nymphs, and in the moulting success of larvae (see SI for details).

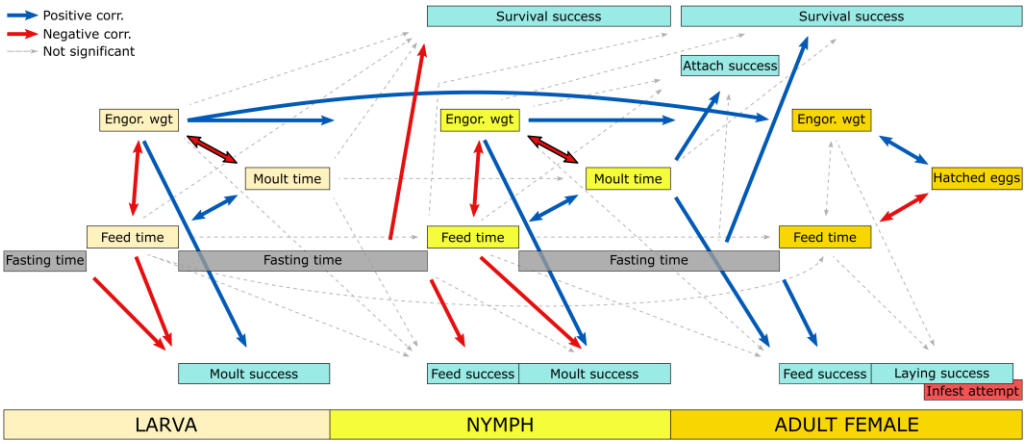

Figure 2. Overview of the main findings for predictors of tick success and correlations between traits. Phenotypic correlations are shown as double-sided arrows and have a black outline if supported by a significant genetic correlation. Single-sided arrows show the effect of predictors on success parameters (light blue). Infestation attempt (infest attempt) refers to adult females attaching at the first or second infestation. Effects are positive (blue), negative (red), or not significant (grey dashed arrows).

Table 2. Predictors of tick success (fixed effects) in Bayesian generalized linear mixed models: means and 95% credible intervals (squared brackets). Significant results are in bold. Not included in the table is the negative effect of infestation attempt on egg-laying success (estimate: -1.54; 95% CI: -4.15, -0.21). Year effects are not shown as these were not significant for any success trait. Full model results are shown in the SI.

Success trait	Feeding time	Engorgement weight	Moulting time	Fasting time
Attachment				
Adult females	0.10 [-0.27; 0.47]	-0.08 [-0.45; 0.29]	**0.41 [0.03; 0.86]**	0.36 [-0.13; 0.86]
Feeding				
Nymphs	-0.04 [-0.18; 0.09]	0.05 [-0.10; 0.20]	-0.00 [-0.15; 0.15]	**-1.73 [-2.03; -1.45]**
Adult females	0.11 [-0.26; 0.48]	0.13 [-0.23; 0.50]	**0.37 [0.00; 0.79]**	**0.59 [0.08; 1.12]**
Moulting				
Larvae	**-0.46 [-0.59; -0.34]**	**2.38 [2.19; 2.58]**		**-0.40 [-0.75; -0.04]**
Nymphs	**-3.31 [-5.60; -1.94]**	**1.52 [0.63; 2.70]**		-0.38 [-1.46; 0.51]
Survival				
Nymphs	-0.07 [-0.20; 0.07]	0.07 [-0.08; 0.21]	-0.01 [-0.16; 0.13]	**-1.64 [-1.93; -1.36]**
Adult females	-0.02 [-0.47; 0.42]	0.13 [-0.30; 0.59]	0.41 [-0.04; 0.91]	**0.68 [0.08; 1.37]**
Egg-laying				
Adult females	-0.48 [-2.03; 0.80]	-0.85 [-2.95; 0.48]		

Phenotypic correlations among traits

At the phenotypic level (Table 3), engorgement weight was negatively correlated with feeding time (Fig. 3) and moulting time in larvae and nymphs, while feeding time increased with moulting time (Fig. S3). In adult females, the number of hatched eggs increased with engorgement weight, while it decreased with feeding time (Fig. 4). Engorgement weight was unrelated to feeding time.

Engorgement weight was phenotypically correlated across all stages, especially between nymphs and adult females ($\tau = 0.460$; Table S8, Fig. S4), while there were no across-stage correlations for feeding time nor moulting time.

Table 3. Phenotypic (above the diagonal) and genetic (below the diagonal) correlations between traits within each life stage with 95% confidence intervals (round brackets) and 95% credible intervals (squared brackets) respectively. Signs were reversed for genetic correlations involving transformed data so that they correspond to the signs for the untransformed data. Transformed data were also used for the phenotypic correlations (similar results with non-transformed data). $^{*}P < 0.05$, $^{**}P < 0.001$.

	Feeding time	Engorgement weight	Moulting time/ hatched eggs[a]
Feeding time			
Larvae		-0.122 (-0.147, -0.097)**	0.079 (0.046, 0.113)**
Nymphs		-0.204 (-0.266, -0.141)**	0.245 (0.182, 0.305)**
Adult females		0.013 (-0.117, 0.139)	-0.155 (-0.297, -0.007)*
Weight			
Larvae	-0.458 [-0.992, 0.330]		-0.259 (-0.283, -0.234)**
Nymphs	-0.060 [-0.756, 0.715]		-0.217 (-0.270, -0.163)**
Adult females	0.276 [-0.459, 0.969]		0.147 (0.016, 0.276)*
Moulting time			
Larvae	0.390 [-0.411, 0.993]	-0.643 [-0.978, -0.229]*	
Nymphs	0.339 [-0.366, 0.944]	-0.389 [-0.823, -0.008]*	
Hatched eggs			
Adult females	-0.099 [-0.877, 0.752]	0.410 [-0.351, 0.971]	

[a]Moulting time replaced by "Hatched eggs" for adult female ticks.

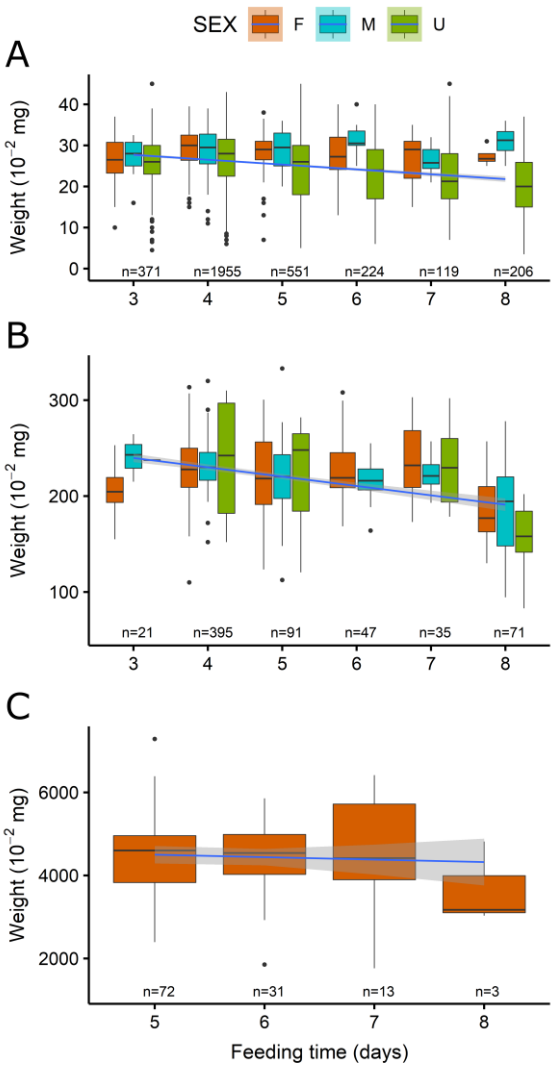

Figure 3. Phenotypic correlation between feeding time and engorgement weight for larvae (A), nymphs (B), and adult females (C). The blue line represents the linear regression fitted to these data with 95% confidence intervals in grey. Females (F) are shown in red, males (M) in light blue, and ticks of unknown sex (U) are shown in green.

Evolutionary potential and genetic correlations

Mean heritability and 95% CI for feeding time, engorgement weight, moulting time (larvae and nymphs), and number of hatched eggs (adult females) are shown in Table 4 along with h_o^2, CV_a, CV_r, and I_a (nymphs). Since models on larvae with and without the sex effect led to highly similar results (Table S9, S10 and Fig. S5, S6) only the model with sex effect is reported below. In larvae, heritability is highest for moulting time (h^2 = 0.133), followed by engorgement weight (h^2 = 0.094) and feeding time (h^2 = 0.043). In nymphs, heritability estimates are overall higher but 95% CI are

also slightly wider. In particular, heritability is substantial for nymph engorgement weight (h^2 = 0.385) and moulting time (h^2 = 0.286). Feeding time is again the trait with the lowest heritability (h^2 = 0.070). Heritability for adult females shows even higher estimates, but also very wide 95% CI for all traits. The shape and mode of the posterior densities for heritability show that in all life stages estimates were clearly different from zero for engorgement weight and moulting time (Fig S9). On the contrary, heritability for feeding time and number of hatched eggs may not be reliable. While heritability of nymph engorgement weight was slightly higher than for moulting time, moulting time had the highest evolutionary potential (CV_a = 25.305, I_a = 0.064), followed by engorgement weight (CV_a = 10.214, I_a = 0.010) and feeding time (CV_a = 5.965, I_a = 0.004; Table S13).

Significant negative genetic correlations were found between moulting time and engorgement weight in both larvae and nymphs (Table 3). The remaining genetic correlations had generally high estimates, but also very wide 95% CI and were not considered significant.

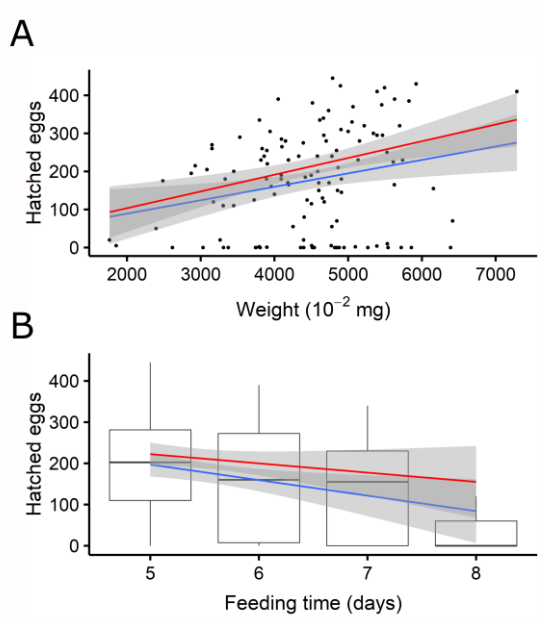

Figure 4. Correlation between number of hatched eggs and engorgement weight (A) and feeding time (B). Blue lines represent the linear regression with adult females with zero eggs included or excluded (red line) and 95% confidence intervals in grey.

Table 4. Fixed effects, components of phenotypic variation (accounting for fixed effects) and heritability estimates for every life stage for four tick traits (Animal Models). Feeding time and moulting time were raised to a negative exponent. Hence, effects have their signs reversed for these traits. In square brackets, 95% lower and upper credible intervals. Fixed effects not overlapping zero were considered statistically significant (in bold). Components of phenotypic variation range between 0 and 1 by definition.

	Feeding time	Engorgement weight	Moulting time	Hatched eggs
Fixed effects:				
Intercept				
Larvae	**0.68 [0.09; 1.27]**	0.27 [-0.24; 0.74]	0.19 [-0.48; 0.74]	
Nymphs	**0.63 [0.19; 1.07]**	0.16 [-0.34; 0.62]	0.16 [-0.45; 0.74]	
Females	-0.44 [-2.08; 1.17]	-0.75 [-2.18; 0.71]		-1.90 [-3.89; 0.06]
Sex:Male				
Larvae	-0.01 [-0.16; 0.12]	0.04 [-0.10; 0.19]	**-0.19 [-0.34; -0.05]**	
Nymphs	**-0.25 [-0.41; -0.10]**	-0.03 [-0.19; 0.13]	**0.17 [0.02; 0.34]**	
Sex:Unknown				
Larvae	**-0.12 [-0.21; -0.04]**	**-0.31 [-0.39; -0.22]**	**-0.10 [-0.19; -0.01]**	
Nymphs	**-1.44 [-1.71; -1.18]**	**-1.21 [-1.50; -0.95]**	1.07 [-0.93; 3.12]	
Fasting time				
Larvae	**-0.006 [-0.010; -0.002]**	-0.000 [-0.003; 0.003]	-0.002 [-0.006; 0.002]	
Nymphs	**-0.005 [-0.009; -0.000]**	0.000 [-0.005; 0.005]	-0.003 [-0.008; 0.002]	
Females	0.005 [-0.016; 0.025]	0.003 [-0.015; 0.021]		0.007 [-0.012; 0.025]
Year:2018				
Females	-0.12 [-0.60; 0.35]	**0.80 [0.45; 1.17]**		1.05 [-0.38; 2.43]
Year:2019				
Females	0.026 [-0.55; 0.59]	**0.73 [0.26; 1.19]**		**1.59 [0.21; 3.07]**
Components of phenotypic variation:				
Additive genetic variance (V_a)				
Larvae	0.050 [0.000; 0.145]	0.100 [0.000; 0.188]	0.152 [0.000; 0.302]	
Nymphs	0.062 [0.000; 0.185]	0.383 [0.099; 0.674]	0.334 [0.120; 0.538]	
Females	0.367 [0.000; 0.811]	0.657 [0.116; 1.166]		0.321 [0.000; 0.802]
Tick clutch effect				
Larvae	0.014 [0.000; 0.052]	0.014 [0.000; 0.042]	0.038 [0.000; 0.096]	
Nymphs	0.049 [0.000; 0.122]	0.061 [0.000; 0.168]	0.032 [0.000; 0.092]	
Females	0.241 [0.000; 0.462]	0.042 [0.000; 0.143]		0.054 [0.000; 0.192]
Feeding event				
Larvae	0.158 [0.084; 0.238]	0.039 [0.010; 0.071]	0.052 [0.008; 0.103]	
Nymphs	0.102 [0.041; 0.171]	0.082 [0.031; 0.141]	0.052 [0.004; 0.101]	
Females[a]	0.136 [0.000; 0.362]	0.048 [0.000; 0.167]		0.067 [0.000; 0.227]
Nest Identity				
Females	0.076 [0.000; 0.213]	0.040 [0.000; 0.131]		0.083 [0.000; 0.245]

Batch				
Larvae	0.228 [0.046; 0.501]	0.202 [0.037; 0.461]	0.273 [0.051; 0.580]	
Nymphs	0.016 [0.000; 0.067]	0.020 [0.000; 0.079]	0.174 [0.021; 0.388]	
Residuals				
Larvae	0.557 [0.348; 0.722]	0.651 [0.438; 0.827]	0.504 [0.273; 0.702]	
Nymphs	0.762 [0.616; 0.896]	0.452 [0.250; 0.657]	0.455 [0.268; 0.633]	
Females	0.250 [0.001; 0.643]	0.272 [0.001; 0.657]		0.489 [0.061; 0.884]
Heritability (h^2):				
Larvae	0.043 [0.000; 0.127]	0.094 [0.000; 0.184]	0.133 [0.000; 0.272]	
Nymphs	0.070 [0.000; 0.207]	0.385 [0.129; 0.649]	0.286 [0.108; 0.461]	
Females	0.298 [0.000; 0.618]	0.597 [0.187; 0.955]		0.308 [0.000; 0.690]

[a]Nestling identity in adult females. The remaining variance of feeding event is explained by nest identity.

Clutch, feeding event, and batch effects

We found that clutch, feeding event, and batch effects were rather small (<10% of the remaining phenotypic variation; Table 4, Fig. 5), with some notable exceptions. Among-clutch variation (representing maternal effects) was high for feeding time in adult females (24.1%, Fig. S10) though with wide 95% CI, while it had low (if any) effect on all other traits and stages. The effect of feeding event was moderate on larval and nymphal feeding time (15.8% and 10.2% respectively) and comparable to its analogue effect (nestling identity: 13.6%) in adult females though the latter estimate had very wide credible intervals. Low, but significant, effects of feeding event were also found for engorgement weight and moulting time in larvae and nymphs (3.9 – 8.2%). Lastly, the batch effect was consistently high for all three larval traits (20 – 27%) and moderate for moulting time in nymphs (17.4%).

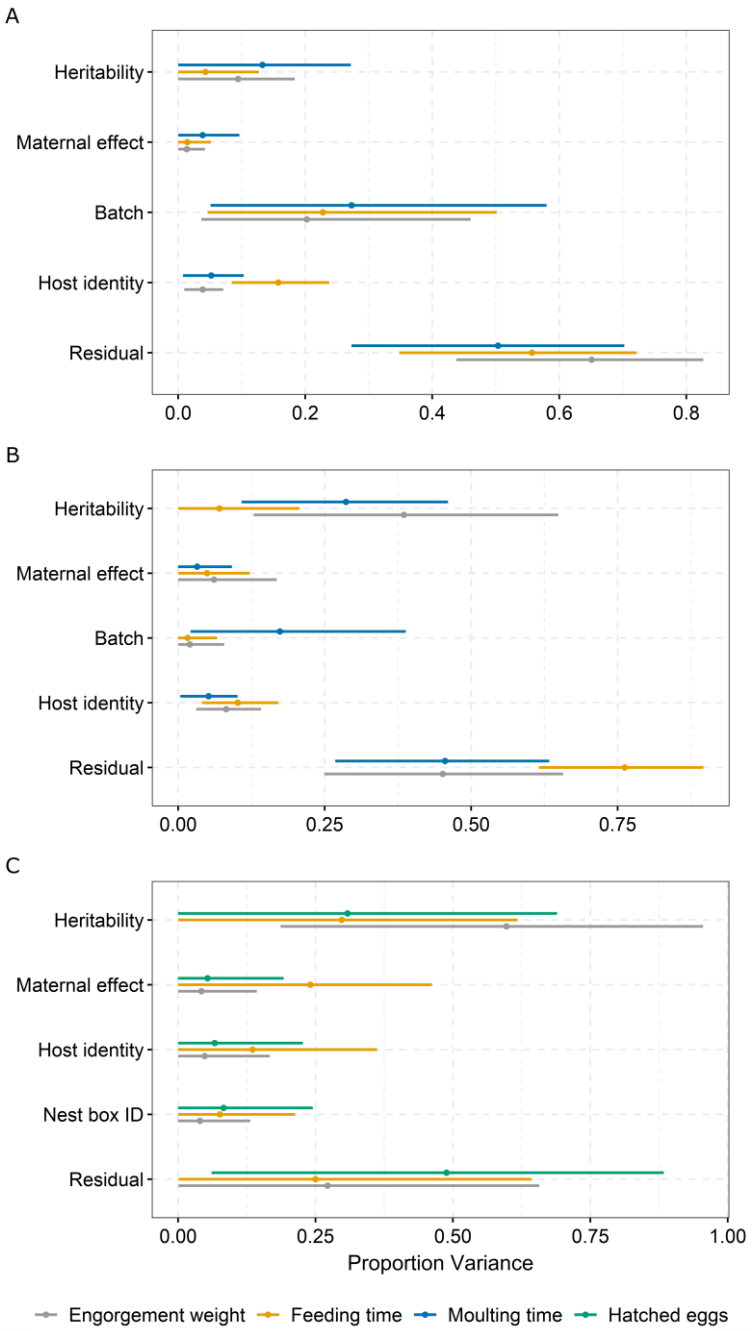

Figure 5. Posterior means and 95% credible intervals of heritability and other components of variation, expressed as proportions of total phenotypic variance after accounting for fixed effects. Panels represent output for larvae (A), nymphs (B), and adult females (C).

Sex, year and fasting time effects

Means and 95% CI for sex, year and fasting time effects are shown in Table 4. Male larvae had significantly longer moulting times than females, but in nymphs this difference was reversed. Also, males had longer feeding times in nymphs, while there was no difference in larvae. Larvae and nymphs of unknown sex differed in multiple traits from females and males probably reflecting associations between these traits and drivers of survival and feeding success. Larvae and nymphs with a longer fasting time, had a longer feeding time, but there was no such association in adult females. Lastly, more eggs hatched in 2019 and adult female engorgement weight was lower in 2017.

Discussion

In this study, we show first of all that variation in life-history traits not only affected parasite success in the same stage, but also had carry-over effects to the next stage in a hematophagous ectoparasite. Second, we found significant trait correlations both within (genetic and phenotypic) and across (phenotypic) life stages. Third, we found significant heritability and evolvability for several traits, notably moulting time and engorgement weight. Fourth, a substantial amount of phenotypic variation in life-history traits could be attributed to tick sex, year, and fasting time as well as to the environmental effects related to host quality (feeding event), shared host physiological responses to the environmental conditions (batch), and tick maternal effects.

The results discussed below should be taken as representative of the life history and evolutionary potential of an *I. arboricola* meta-population at a regional scale rather than of a single population. Our founder set of ticks was in fact collected from four wooded plots spread over a 50-km wide area. A previous study showed moderate genetic differentiation between these areas (Van Oosten et al. 2014a), but since they were located in quite similar habitat (mature oak-dominated woodland)

and similar host communities, adaptive genetic differentiation between plots was probably rather limited.

With regard to the predictors of tick success, both attachment and feeding success in adult females were increased when nymphs took longer to moult to adults. Interestingly, there was no effect of larval moulting time on nymph feeding success suggesting stage-specific differences between nymphs and adults. On the other hand, larval and nymph moulting success were both increased when feeding times were short and engorgement weights high, which we will discuss further in the next paragraph. Somewhat surprisingly, no trait predicted overall survival to the next life stage in nymphs nor females. We suggest this might be due to the wide number and different nature of selective pressures acting on ticks.

Unsurprisingly, ticks with a higher blood intake increased their chances to moult to the next stage, as previously found in the lone star tick *Amblyomma americanum* (Koch 1986). The negative correlation between engorgement weight and feeding time in both nymphs and larvae indicates that longer feeding does not necessarily imply a higher blood intake, and therefore does not support the hypothesis of a trade-off between resource acquisition and exposure to host defences. A similar negative correlation between engorgement weight and feeding time was shown in adult female *R. appendiculatus* (Wang et al. 2001). These results rather suggest individual differences in the rate of blood intake: some ticks need more time to complete engorgement, and even end up with lower weight. In spite of a somewhat complex relationship between the different success parameters, our findings suggest that the optimal feeding time for larvae and nymphs overlaps with, or is close to, the shortest feeding time, namely 3 – 4 days, while we saw no relation between feeding time and fitness for adult ticks. It can be hypothesized that ticks that fed for longer and eventually reached a lower engorgement weight were less well adapted to the host species, as was observed for different host races in *Ixodes uriae* (Dietrich et al. 2014). This seems rather unlikely in our case, as the main hosts of *I. arboricola* in our study areas are great and blue tits which are closely related and

share the same roosting and breeding cavities; records on other cavity-nesting birds in the region are very scarce (Van Oosten et al. 2014a). This ecological context would therefore hamper the evolution of host-specialized races. The site of attachment may also have contributed to differences in feeding time and engorgement weight if attachment sites differ in blood flow or inflammatory response. However, according to the natural attachment behaviour of ixodid ticks on birds (Fracasso et al. 2019) and our infestation protocol, we are confident that the vast majority of *I. arboricola* attached to the bird's head possibly reducing the differences between feeding sites. Alternatively, Wang et al. (2001) hypothesized competition among ticks mediated by the host as a possible driver of such variation: fast-feeding ticks might exacerbate the host's immunological response, which has a comparatively stronger effect on slow-feeding ticks reducing their blood intake further. However, this hypothesis is unlikely here as we did not find signs of anti-tick immunological resistance in our host (Heylen et al. 2021; Heylen et al. 2010). The hypothesis that engorgement weight reflects individual quality is confirmed when looking at moulting time: intuitively, engorgement weight and moulting time should be positively correlated since a bigger blood meal should take more time to be processed, as observed across stages (Heylen et al. 2014c). Instead, we found that within the same stage, ticks with higher engorgement weights and shorter feeding times needed less time to moult. For adult females, we show that engorgement weight is a strong predictor of fitness as measured by the number of hatched eggs (see also Van Oosten et al. 2016a), in accordance with other tick species (Ginsberg et al. 2016; Gray 1981; Ma et al. 2013). Though we did not find any significant correlation between adult females' feeding time and engorgement weight (possibly due to a higher measurement error for feeding time), the former was again negatively correlated to the number of hatched eggs. It is important to point out that results related to engorgement weight may be partly underpinned by variation in body size, whereby morphologically larger ticks may have reached higher engorgement weight. Our data do not allow us to distinguish between tick size and the amount of blood ingested. While this would

definitely be interesting from a fundamental point of view, engorgement weight per se is a highly relevant trait in the context of host exploitation since it directly relates to the amount of resources extracted from the host. Anyway, our results support the hypothesis of variation in the parasite's individual quality for which engorgement weight seems to be a good proxy.

In all cases, the genetic correlations were in the same direction as the phenotypic correlations, although only moulting time and engorgement weight were significantly correlated at the genetic level. This may be due to a functional (pleiotropy) or spatial linkage (linkage disequilibrium) between the genetic pathways involved in feeding, body size, and metamorphosis (Armbruster and Schwaegerle 1996; Saltz et al. 2017). As we already discussed, longer moulting times as nymphs are associated with higher attachment and feeding success at least in adult females, although we have no explanation for the underlying mechanism. Hence, ticks could hypothetically trade off a low engorgement weight, with long feeding and moulting time, for a higher attachment success. Thus, our findings do not exclude the existence of alternative life-history strategies that maximize the likelihood of attachment (survival) versus the number of offspring, though this hypothesis would need to be further investigated.

We found significant correlations between engorgement weight, a key predictor of individual tick success, across the three developmental stages. This is to our knowledge the first evidence of across-stage maintenance of individual variation in a key life-history parameter in a parasite. This correlation could be underpinned by differences in tick size, but further studies are needed to investigate such hypothesis. The adaptive decoupling hypothesis posits that separate life stages should allow for independence and adaptation of each stage to specific tasks (Ebenman 1992). Although across-stage trait correlation is still poorly understood, complete stage independence is never realized, as we show in our study, due to the sharing of the same genome, ontogenetic pathways, and correlated changes in selective pressures (Benesh 2016; Thia et al. 2018). Such correlation between stages might originate

from intrinsic genetic variation (i.e. differences in tick quality) in factors determining engorgement weight, such as feeding efficiency or inherent body size, or be due to a host compatibility mainly determined in the larval stage. In the latter case, larvae feeding on high-quality hosts will have positive carry-over effects later in life.

To our knowledge, this is one of the first studies showing heritable variation in a measure of host exploitation (see also Madder et al. 1996). In fact, engorgement weight and moulting time show substantial estimates of evolvability and heritability across the three stages, and thus have the potential to evolve rapidly under changing selective pressures. While longer moulting time is traded-off with higher engorgement weight, we did not find any direct cost for the latter raising questions on the maintenance of its genetic variation. However, our study does not allow to disentangle the contributions of intrinsic (morphological) size variation and blood meal size. Conversely, evolutionary changes in feeding time are less likely to occur. Due to its lack of evolutionary potential and its correlations with the other traits, feeding time seems to be a more flexible life-history parameter, adjusted by ticks based on their phenotypic quality and/or tick-host compatibility. Interestingly, nymphs show higher heritability than larvae for all traits. This might be partially explained by the study design, where each bird was infested by only a single larval clutch, but with nymphs from multiple clutches which allowed for an improved distinction between feeding event versus clutch identity. Alternatively, heritability in larvae may be lower due to a higher sensitivity to environmental variation. Although adult females had higher heritability compared to larvae and nymphs (range 30 – 60%), the comparison with the other stages should be done with caution due to the very wide credible intervals. Similarly, more data are needed to assess if adult female feeding time has any heritable component. With regard to maternal effects, they were generally low and in line with the effect size of maternal effects for life-history traits (<10% of total phenotypic variation) measured from 151 studies of animal populations (Moore et al. 2019). Compared to natural conditions, maternal effects

may have been minimized in our set-up due to the standardized feeding of adult ticks on nestlings of the same species, similar age and size.

Host identity as estimated by the effect of feeding event had a low/moderate but significant effect on tick trait variation, affecting all larval and nymph traits. We thus provide evidence that host characteristics affect expression of parasite traits, a fundamental requirement for host-parasite co-evolution (Clayton et al. 2015). Investigating the sources of host variation is outside the scope of this study and will be reported elsewhere in more detail. Briefly, this variation could originate by intrinsic (permanent) host variation, by host condition at the moment of infestation (aside from batch effects common to all individuals, as described below), but also by variation in actual tick feeding density, i.e. between birds in the number of ticks that actually attached (Bartosik and Buczek 2012; Van Oosten et al. 2016b; Wang et al. 2001). Surprisingly, batch effects were considerable despite highly standardized conditions. These may be attributed to between-batch differences in host physiology and behaviour that affected tick parameters as in earlier studies (Bize et al. 2008; Seppälä et al. 2008; Tschirren et al. 2007). Specifically, the shared conditions experienced before infestation by birds of the same batch (e.g. the degree of contrast between natural and indoor environments at capture) may have persisted in controlled lab conditions, shaping this batch effect. On average, between-batch variation was higher in larvae maybe due to their higher environmental sensitivity. Differences between batches due to tick physiology (e.g. motivation to feed) also cannot be ruled out completely. Nevertheless, our experimental infestations matched the normal seasonal feeding activity of ticks in the wild, as larvae and nymphs of *I. arboricola* feed throughout the year, while adult females mostly feed in the nestling period (Heylen et al. 2014c). Moreover, our models accounted for variation in fasting time between infestations, although it remains possible that part of the between-batch variation in fasting time could have contributed to batch effects.

We found sexual differences in both feeding and moulting time. In particular, male larvae moulted more slowly, while male nymphs fed for longer and moulted faster than females. Intriguingly, male nymphs of *Amblyomma maculatum* also fed longer than females despite a lower engorgement weight (Nagamori et al. 2019). On the contrary, *I. ricinus* female nymphs had a longer feeding and moulting time as well as engorgement weight (Dusbébek 1996). Despite the lack of a consistent pattern, such findings show that sexual differences in tick life-history traits go well beyond differences in engorgement weight and act already before the adult stage. Sex-specific selective pressures can thus take place at an early stage. However, it is worth mentioning that ticks with known sex represent the subset of ticks that survived until maturity. Unsurprisingly, ticks of unknown sex had lower engorgement weight and longer feeding time compared to males and females, both associated with reduced survival.

As in our experimental design not all ticks could feed at the same time, we could investigate the effect of fasting time (range: 5 – 155 days) which had multiple and sometimes opposite effects. A longer interval between feeding events affected overall survival negatively in nymphs and positively in adult females. It was further associated with lower moulting success in larvae, lower feeding success in nymphs and higher feeding success in adult females. Also, longer fasting time led to longer feeding time in both larvae and nymphs. Overall, long-fasting larvae and nymphs seem to have lower fitness while we found the opposite for adult females. This remarkable difference may partially be explained by the higher tolerance of adult females to fasting and environmental stresses (Campbell and Glines 1979; Chilton and Bull 1993; Newson et al. 1984; Rosendale et al. 2017; Tsunoda 2008) although it does not explain a reversal in the direction of the effect. In any case, we show that the time elapsed between feeding events played an important role in the life history of a hematophagous ectoparasite and therefore should be taken into account in future studies.

Conclusions

In the light of our findings, we hypothesize that variation in fitness in this hematophagous ectoparasite feeding on its main host is mainly affected by individual differences in quality. Such differences are expressed in both engorgement weight and feeding time, although we cannot exclude the presence of alternative life-history strategies (optimizing attachment success or offspring number). Genetic variation and carry-over effects can both account for the variation in tick quality. Furthermore, we show that key life-history traits such as engorgement weight and moulting time have the potential to respond to selection. Tree-hole ticks might thus be able to adaptively adjust their feeding strategies and exploitation of hosts, contributing to a dynamic host-parasite interaction. Individual-based studies are promising, but under-exploited tools to investigate the selective pressures and evolutionary potential of parasite traits. Here, we use this approach and show how non-permanent ectoparasites are good model systems that overcome many of the limitations of other parasites, enabling us to obtain a deeper understanding of host-parasite interactions.

Chapter IV

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

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Abstract

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 an 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.

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 (Bize et al. 2008; Christe et al. 2003; Heylen and Matthysen 2011b). 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; Izhar and Ben-Ami 2015; Lourenço and Palmeirim 2008), sex (Roberts and Hughes 2015; Sanchez et al. 2011), 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 (Jones et al. 2015; Tschirren et al. 2007). 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 (Clayton et al. 2015; Gagneux 2012; Masri et al. 2013; 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 (Mazé-Guilmo et al. 2014; Smith et al. 1999). 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 (Hemingway et al. 2016; Nauen 2007; 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 (Bush et al. 2019; Dlugosz et al. 2014). 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 (Clayton et al. 2015; Clutton-Brock and Sheldon 2010). In the wild, most ectoparasite species are unevenly distributed on hosts, with few hosts contributing to most of the parasite population (Clayton et al. 2015; Clayton and Moore 1997; Poulin 2007). 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. 2014c; Van Oosten et al. 2018; Van Oosten et al. 2014a; Van Oosten et al. 2016b). Even

though *Ixodes arboricola* has negligible effects on host health (see 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 (Fig. S1-S3; Heylen et al. 2014c). 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 2011a; Van Oosten et al. 2016b). 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. 2021; Heylen et al. 2010) – 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 (Fig. 1 for an overview). 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 performance among hosts could be explained by host sex, age, body condition (and its change over the captivity period) and haematocrit.

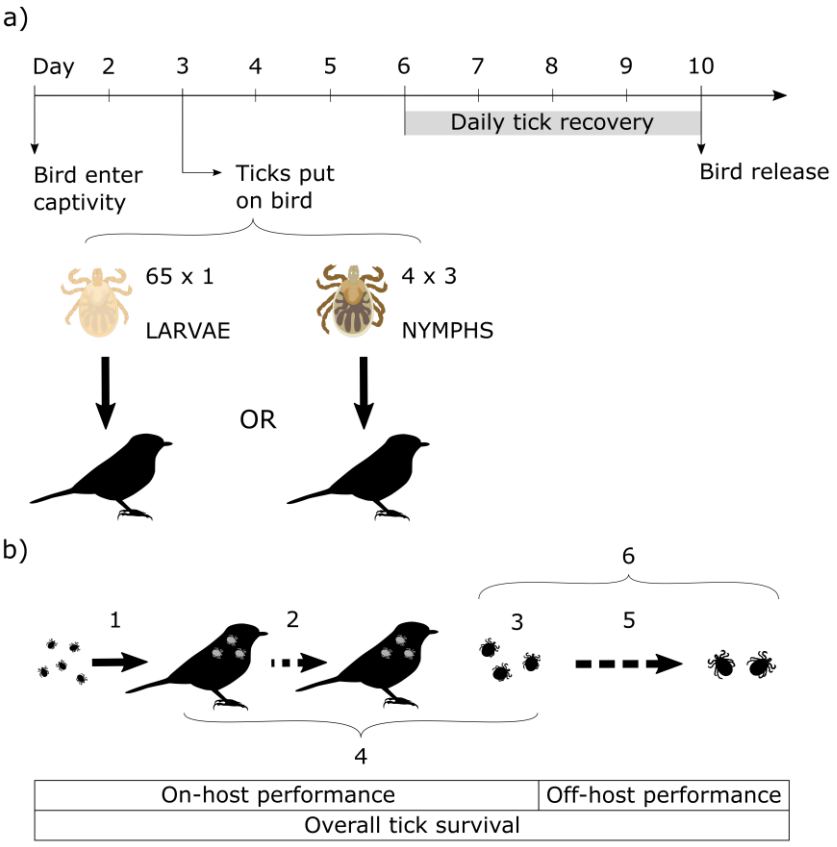

Figure 1. Study design with definition of tick variables. a) Overview of the experimental infestations on adult great tits. After two days of acclimatization every bird was infested with either 65 larvae from one clutch or 12 nymphs from 3 clutches (4 ticks from each clutch). b) Overview of tick performance 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.

Material and methods

Study species

Between 2017 and 2020 two consecutive generations of the nidicolous tree-hole tick *Ixodes arboricola* (Schulze and Schlottke, 1929) were reared in laboratory conditions and fed on wild great tits *Parus major* Linnaeus, 1758. 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 (see Fracasso et al. 2022a). 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* (Heylen et al. 2014c; Sonenshine and Roe 2013; Van Oosten et al. 2014b; White et al. 2012). Immature *I. arboricola* stages naturally feed throughout the year whenever birds use cavities (Heylen et al. 2014c).

Great tits are small songbirds preferentially breeding in deciduous woodlands and widespread across Europe, part of Asia and North Africa (Cramp et al. 1993). The birds used in this study were part of a wild population settled in the Boshoek area (51° 7' 59" N, 4° 31' 1" E) near Antwerp (Belgium) and breeding in nest boxes (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 abovementioned one) within a 25 km distance from the centre of Antwerp to establish a lab population (Van Oosten et al. 2014a). Two consecutive complete tick generations were raised in semi-natural conditions and individually followed throughout their three life stages (see also Fracasso et al. 2022a). 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 hours 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 et al. 2017; Heylen and Matthysen 2010; Heylen et al. 2014b). 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 (Fracasso et al. 2019; Heylen et al. 2017). Nymphs were individually marked by clipping part of one limb (except the first pair holding the Haller’s organ) with a scalpel within 2 hours 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 5 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 (scale: Mettler Toledo XS205) 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: i) at capture, ii) on the fourth day after infestation (i.e. at the peak of tick detachment), iii) at release. Body condition was expressed relative to tarsus length using the scaled mass index, for males and females separately (Peig and Green 2009; Peig and Green 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 (11468 males and 10645 females). Previous studies have shown that bird body condition is related to survival (Krams et al. 2010; Naef-Daenzer et al. 2001), immune response (Bowers et al. 2014; Navarro et al. 2003), 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 et al. 1993).

Before bird release, a blood sample was taken using a heparinized capillary (60 µl) 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 minutes 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 2011a). 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 4.0.5 (R Core Team 2020). To check model assumptions we plotted the distribution of the standardized deviance residuals and checked for the presence of outliers using the "DHARMa" (v. 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:

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

where $\mathbf{y}$ is the response variable as a N × 1 vector (in our study a tick performance variable); $\mathbf{X}$ is a N × *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 × *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" (v. 2.15.0) package (Bürkner 2017; Bürkner 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" (v. 1.7.0) package (Peterson and Cavanaugh 2020). 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 (Fig. S4, S5) and estimates of the additive genetic and residual variance (Table S12) 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" (v. 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. 2022a; Van Oosten et al. 2016b). 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 (Table S1, S2 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 (Fig. S4 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.

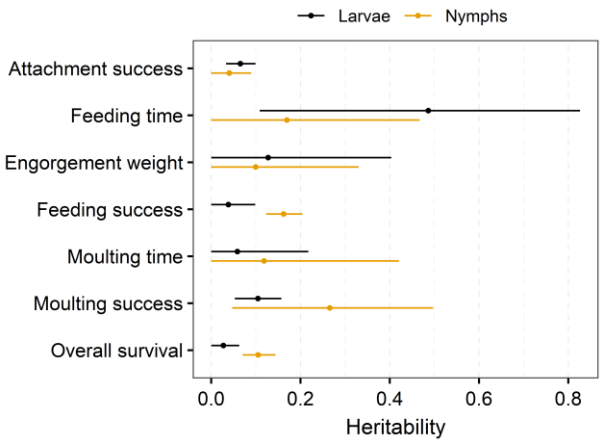

Figure 2. Strength of the influence of host genetic background (host heritability) on performance variables of larvae (black) and nymphs (yellow). Dots and horizontal lines show mean estimates and 95% credible intervals respectively.

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, std. err. = 0.136, z = 3.74, $P < 0.001$, Fig. 3; survival estimate: 0.426, std. err. = 0.137, z = 3.11, $P = 0.002$, Fig. 4). Also, a higher proportion of nymphs survived on older birds (estimate: 0.202, std. err. = 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, std. err. = 0.028, t = -3.02, $P = 0.003$).

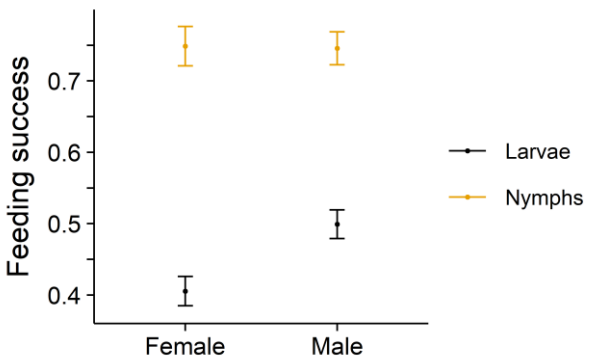

Figure 3. Mean observed feeding success (proportion of recovered ticks out of attached ones) in male and female great tits for larvae (black) and nymphs (yellow). Error bars represent ±1 standard error of the mean. In larvae, sex differences were statistically significant in the respective GLMM.

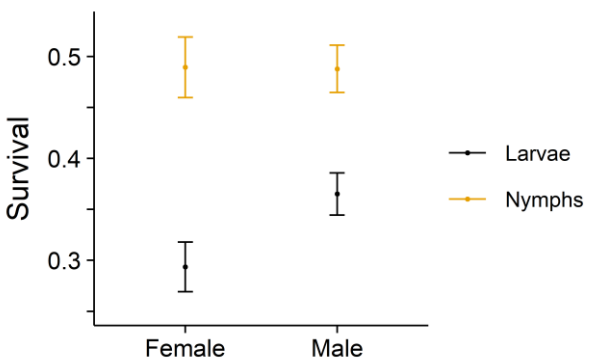

Figure 4. Mean observed overall survival (from infestation to moulting into the next stage) in male and female great tits for larvae (black) and nymphs (yellow). Error bars represent ±1 standard error of the mean. In larvae, sex differences were statistically significant in the respective GLMM.

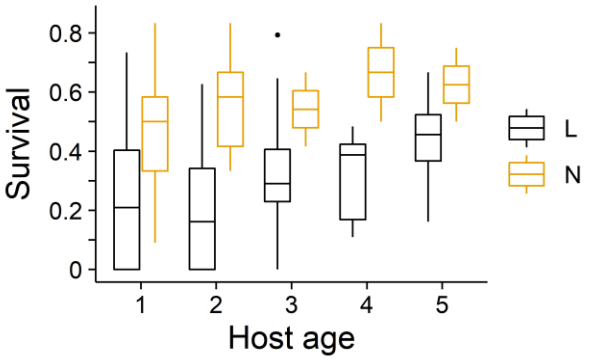

Figure 5. Mean observed overall survival (from infestation to moulting into the next stage) of larvae (L, black) and nymphs (N, yellow) put on great tits of different age (in years). Median (horizontal lines), interquartile range (box limits) and potential outliers (dots) for every age class are shown. The only 6-year old host is not shown for visual clarity. In nymphs, age differences were statistically significant in the respective GLMM.

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, std. err. = 0.031, t = 3.51, $P < 0.001$; moulting time estimate: 0.143, std. err. = 0.050, t = 2.83, $P = 0.006$). Feeding density did not significantly affect any tick performance variable, neither in larvae nor in nymphs.

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)

In addition to the abovementioned correlations our results also possibly suggest ($0.01 < P$-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, S1, S2) 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.

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. ^{a}Blood-sampled subset (see main text).

	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.

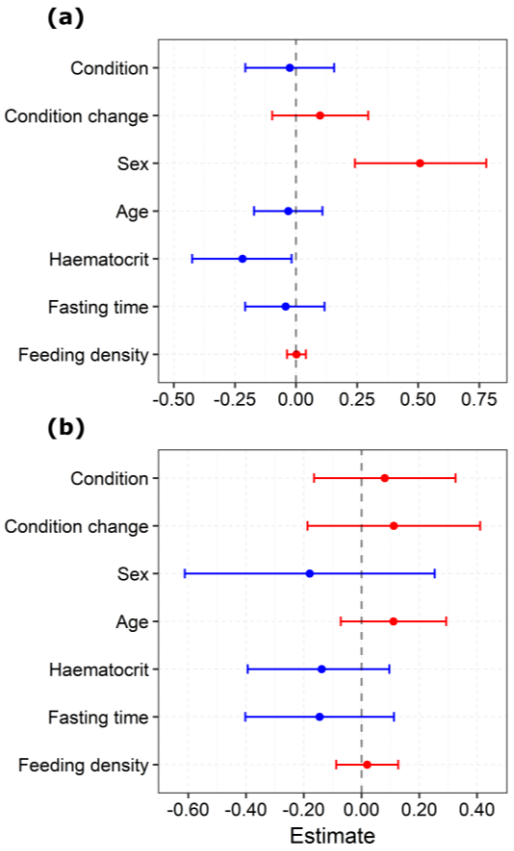

Figure 6. Mean effect size and 95% confidence intervals of host traits on feeding success (proportion of recovered ticks out of attached ones) of larvae (a) and nymphs (b). Positive estimates are in shown in red, negative estimates in blue.

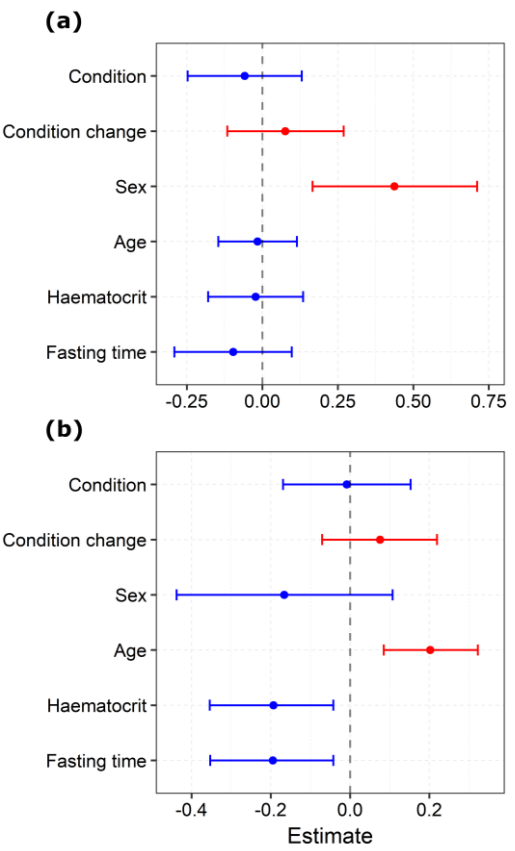

Figure 7. Mean effect size and 95% confidence intervals of host traits on overall survival (from infestation to moulting into the next stage) of larvae (a) and nymphs (b). Positive estimates are in shown in red, negative estimates in blue.

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.

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 naturally infested 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 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 et al. (2013a) 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. 2013a) 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 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 (Fracasso et al. 2022a). 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 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). 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 (Roberts et al. 2004; Ruiz-Fons et al. 2013; Tschirren et al. 2003). 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; Klukowski and Nelson 2001; Poiani et al. 2000). 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. 2013a). 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 (Izhar and Ben-Ami 2015; Izhar et al. 2015; Lawrence et al. 1976) 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 (Christe et al. 2000b; Fair et al. 2007; Potti et al. 1999; Shlosberg et al. 1998), 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, nor its subsequent change during captivity affected tick performance. However, the relationship between the change in body condition and larval attachment success deserves further investigations. 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. 2017b). 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 acari, 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. 2021; Heylen et al. 2010). 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 (Lloyd-Smith et al. 2005; Ostfeld and Keesing 2000; 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.

Chapter V

Male mating preference in an ixodid tick

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Abstract

Background: Mate choice is a fundamental element of sexual selection with the potential to shape the evolution of traits. It has been shown that mate choice based on body size is common in several arthropod species. In hard ticks, a taxon of medical and veterinary importance, engorgement weight is positively correlated with reproductive output but it is unknown whether adult males show mate choice. Here, we experimentally investigated whether males i) use chemical cues to choose their mating partner, ii) consistently choose for the same female individual, iii) prefer females with highest weight after feeding.

Methods: We used two experimental setups where chemical communication was allowed: a horizontal tube hindering physical contact with the female, and an arena where tactile cues were allowed. In total, we tested 62 triads composed of one male that could choose between two engorged females. Twenty-four triads were repeatedly tested in the tube and 38 triads were tested in both setups.

Results: We found no preference for individual or heavier females in either setup. However, in the horizontal tube males significantly preferred females that were not visited by them in the previous test.

Conclusions: Our results suggest a lack of male mate choice despite heavier females having higher fecundity. However, future studies should take into account that males may recognize the potential mating partners they previously met.

Introduction

Sexual selection is among the most important evolutionary processes shaping the morphology, behaviour, life history, and ecology of species (Andersson 2019; Hollis et al. 2009). One of the main components of sexual selection is mate choice, i.e. the differential sexual response leading to non-random mating with respect to one or more traits that are displayed in sexually mature individuals of the opposite sex and same species (Bonduriansky 2001). Mate choice alters the reproductive success of individuals (Andersson and Simmons 2006; Jiang et al. 2013; Ritchie 2007) and can evolve through direct and indirect selection on mating preferences. The evolution of mate choice usually occurs in the sex with greater reproductive investment, either the one with greater parental care or that with higher mating effort (Byrne and Rice 2006; Edward and Chapman 2011; Wittman and Fedorka 2015). The evolution of mate choice is influenced by several factors that include (but are not limited to) mating investment, operational sex ratios (i.e. proportion of sexually active males and females), costs and benefits of choosiness, and variation in mate quality (Bonduriansky 2001; Edward and Chapman 2011; Fawcett and Johnstone 2003). For instance, mate choice is favoured when the number of available mates is higher than the capacity to mate (Edward and Chapman 2011). This condition is often satisfied when there is a simultaneous or frequent encounter of potential mating partners (Barry and Kokko 2010; Head et al. 2015). High investment in mating and high variance in the quality of mating partners are also important promotors of mate choice. Several traits can be used to assess mate quality in the context of mate choice. Specifically, a trait used for mate assessment needs to satisfy three criteria (Searcy 1982): i) its expression in the chosen mate influences the fitness of the individual making the choice, ii) there is considerable variation between potential mates for such trait, iii) the trait (or a correlated one) can be reliably evaluated prior to mating.

Historically, mate choice has been mainly investigated in the context of females being the choosy sex. Male mate choice was first proposed to occur in

species with reversed sex roles where males exhibit higher parental care and females compete for them (Eens and Pinxten 2000). However, theoretical studies suggest that male mate choice may occur and evolve under broader conditions than originally thought such as in the absence of male parental care, in presence of more sexually active females than males (i.e. female-biased operational sex ratio), or in polygynous species (Edward and Chapman 2011; Fitzpatrick and Servedio 2018).

Arthropods are an abundant and taxonomically diverse group of organisms where mate choice has been observed in multiple taxa including insects (Arnqvist et al. 1996; Bonduriansky 2001), arachnids (Bel-Venner et al. 2008; Kralj-Fišer et al. 2013) and crustaceans (Fazhan et al. 2017). In this taxon, body size is considered to be one of the most prevalent choice criteria (Bonduriansky 2001; Crespi 1989; Reading and Backwell 2007). Among arthropods, ticks are hematophagous ectoparasites that transmit a large number of diseases (Dantas-Torres et al. 2012; Jongejan and Uilenberg 2004; Parola and Raoult 2001) but little is known on their mating strategies. Since mate choice can play an important role in selecting for a vast array of traits (Andersson 2019), understanding if and how ticks choose their mating partners will help us to comprehend how tick traits can have evolved and diverged between species. In hard ticks mating can occur both before (preprandial) and after (postprandial) feeding. However, literature evidence shows that in some tick species males are more attracted by adult females that are engorging (e.g. in *Ixodes ricinus*), or fully engorged such as in *Ixodes arboricola* (Van Oosten et al. 2016a; Zemek et al. 2002). By mating with engorged females, males avoid the risk of reproducing with ticks that could then fail to find a host and feed. Body size and engorgement status may be assessed through chemical or tactile cues. Visual capabilities beyond the perception of day-night cycle are to be excluded as only few bilaterally arranged photoreceptors have been found in a congeneric tick (Perret et al. 2003).

Some life-history characteristics suggest that mating is costly for males of the genus *Ixodes*. For instance, experimental evidence suggests that prostriate males, contrary to other tick groups, may only be able to inseminate few adult females (Graf

1978; Kiszewski et al. 2001; Yuval et al. 1990). Moreover, prostriate ticks often remain in copula much longer than the time required for sperm transfer (Graf 1978; Kiszewski et al. 2001; Van Oosten et al. 2016a). This form of mate guarding is likely an adaptation to prevent insemination from other males and thus ensuring paternity of the offspring (Alcock 1994). However, postcopulatory mate guarding is costly for males as during this period further reproductions are prevented. Moreover, mate guarding can incur additional costs if a female of poor quality is chosen as mating partner while more fecund females are being fertilized by other males. In these conditions, it can be hypothesized that male mate choice will be favoured by selection.

Ticks are important vectors of diseases and an excellent model system for the study of host-parasite interactions at the individual level (Fracasso et al. 2022a; Fracasso et al. 2022b). Nevertheless, our knowledge about mate choice in this group of ectoparasites is scant. Moreover, evidence shows that different tick-borne diseases such as the Lyme disease caused by *Borrelia* spp. (Rudenko and Golovchenko 2021), the tick-borne encephalitis virus (Chunikhin et al. 1983), and Rickettsiae (Hayes et al. 1980) can be sexually transmitted in ticks. Hence, understanding tick mating behaviour may improve our comprehension of the population and evolutionary dynamics of ticks and the pathogens they vector.

The tree-hole tick *Ixodes arboricola* Schulze & Schlottke is a bird-specialized nidicolous tick that lives in tree holes and nest boxes. Immature stages feed throughout the year mainly on adult birds while adult females feed on nestlings (Heylen et al. 2014c). Several *I. arboricola* characteristics suggest that male mate choice should be favoured in this species: first, this tick has a female-biased sex ratio (Van Oosten et al. 2018) whereby adult females outnumber adult males, further promoting male choice. Second, since adult *I. arboricola* females almost exclusively engorge on nestlings during the breeding season (Heylen et al. 2014c) they synchronize their attachment with the host development (Heylen et al. 2012). In the hosts exploited by the tree-hole tick, all nestlings grow at the same time in the nest.

Hence, every year the majority of tick females likely engorge and detach within a couple of days (Heylen and Matthysen 2011b) thus making them available for mating almost simultaneously and providing the few males with the choice between several females. At this stage females can be fertilized and subsequently lay the eggs. Males that initiate mating in late may have a reduced number of potential partners from which to choose. Third, in *I. arboricola* engorgement weight is highly variable, and strongly positively correlated with the number of hatched eggs (Van Oosten et al. 2016a), as for other tick species (Chen et al. 2009; Ginsberg et al. 2016; Ma et al. 2013). Sexually active males would thus gain fitness benefits from choosing the heaviest engorged female available. Fourth, males exhibit postcopulatory mate guarding behaviour even beyond egg deposition (Van Oosten et al. 2016a). It has been suggested that this is an adaptive strategy to prevent fertilization from other males given the absence of sperm precedence (Van Oosten et al. 2016a). Fifth, due to the female's impaired mobility after feeding, females have little opportunities to refuse the mobile (unfed) male and its mating attempts. However, females could still influence male fertilization success through other physical or chemical mechanisms, i.e. cryptic female choice (Eberhard 1997).

In this study we tested whether i) chemical or tactile cues mediate information on mate choice, ii) *I. arboricola* males are consistent in their preference for individual females, and iii) if males prefer heavier females. To address these questions we used two different experimental setups.

Materials and methods

Ixodes arboricola is an endophilic hard tick (Ixodidae) distributed across the Palearctic region (Liebisch 1996; Petney et al. 2012). It feeds primarily on cavity-nesting birds, in particular great tits *Parus major* and blue tits *Cyanistes caeruleus* (Arthur 1963; Heylen et al. 2014c; Hillyard 1996).

Adult ticks were derived from a breeding lab population that was founded with ticks from four areas to ensure genetic variability (details in Fracasso et al. 2022a).

The immature developmental stages (larvae and nymphs) fed on wild-caught adult great tits that were kept in captivity only for the duration of tick engorgement, while adult females fed on great tit nestlings in nest boxes during the great tit breeding season.

In June 2019, male mating preference was tested in randomly chosen triads consisting of one adult male and two fully engorged female ticks. This was done in two experimental setups with different characteristics (Fig. 1): a circular arena and a horizontal tube. The two setups differed in the amount of tactile information, where females could be touched by the male in the arena but were physically not accessible in the horizontal tube (details are given below). Female chemical volatiles could instead always be perceived by the male in both setups although the arena had a much bigger air volume. The majority of triads was tested in both setups thus allowing to estimate the repeatability of male choice for individual females between setups. In the horizontal tube, a number of triads were also tested twice. Before every trial, the experimental setups were rinsed with 70% Ethanol and left to dry. Gloves were worn by the experimenter who was blind to tick characteristics and previous male choice. Experiments were carried out in low light to mimic natural conditions. The experimental room was air-ventilated thus reducing any odour contamination between trials and experimental setups.

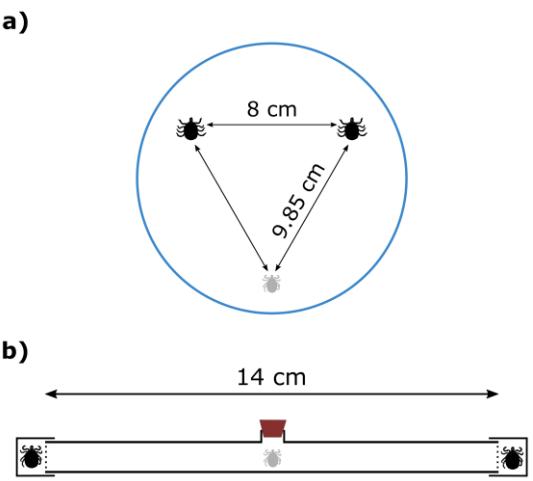

Figure 1. Overview of the study setups. One male (grey) was given the choice between two engorged females (black) in two different setups: an arena (**a**) and a horizontal tube (**b**). In the arena the two females were put upside down, 8 cm apart from each other, while the male was put at a 9.85 cm distance from each female. A plastic dome (blue) prevented ticks from escaping, while keeping the arena free from uncontrolled airflow. In the horizontal tube (**b**) females were put 14 cm apart and their sight was prevented by a plastic mesh (dashed lines). A plastic cork (brown) sealed the entrance of the horizontal tube.

Horizontal tube setup

The horizontal tube allows the male to only use chemical cues to assess potential mating partners. Females were placed at opposite ends of a 14-cm-long glass tube (Fig. 1). The tube was circular with a diameter of 18 mm. A piece of mesh cloth on each side held the female in place preventing physical contact with the male as well as actual mating. Two plastic lids closed both extremities thus preventing unidirectional airflow within the tube. After both females were placed in position a male was put in the centre of the glass tube through a hole subsequently closed with a plastic cork. Males were positioned perpendicularly to the females and their position was recorded every minute for a total of 25 minutes. A female was considered chosen if the male crawled within 1.5 cm from it. The same tube was used for all tests and its position relative to the observer and the room was kept constant throughout the experiment. Mesh cloths and the plastic cork were always rinsed in 70% ethanol and randomly swapped between trials. The total time spent in each arm of the maze was also recorded. Forty-two different triads were tested. Males were chosen not to be siblings of any of the two tested females. To estimate repeatability of male choice, 24 triads were tested a second time one or two days later (median: 2 days) after swapping the females' positions. Males did not show any significant preference for either side of the tube in the first nor second tube test (both $P > 0.175$).

Arena setup

In the arena both tactile and chemical stimuli could be used to assess the mate. A circle of 12-cm diameter on the smooth plastic surface of a table delimited the setup. Inside it two females were placed 8 cm apart from each other leaning on the dorsum to prevent them from moving in the arena. The male was put at a 9.85 cm distance from each of them (Fig. 1). A transparent plastic dome was placed over the arena thus preventing a unidirectional airflow. A female was considered chosen

when the male crawled on top of the female. Each trial ended when a female was chosen or after 50 minutes in case of no choice.

In the arena setup males could initiate copulation soon after female choice, and this could affect subsequent mate choice. Therefore all triads undergoing both experimental setups were first tested in the horizontal tube followed a few days later by the arena test (average: 5 days, range: 4 – 8 days after the last tube test).

In total, we carried out 124 tests investigating 62 different triads with a total of 62 males and 93 different females. In the horizontal tube, 42 triads were tested, of which 24 were repeated once, giving a total of 66 tube tests. In the arena, we carried out 58 tests in total. Specifically, we investigated 46 triads and 12 additional couples consisting of females that were not chosen in the first test. These 12 couples were tested a second time with a different male to estimate choice consistency across males. Thirty-eight triads were tested in both setups (Table 1). Female body weights ranged from 18.56 mg to 72.88 mg. The average weight difference between females in a triad was 10.71 mg in the horizontal tube and 10.85 mg in the arena (range in both cases: 0.49 – 35.56 mg).

Table 1. Sample size of the individuals and tests carried out in the horizontal tube, arena, and in both setups. We tested the choice of every male between two different females, with every combination of male and females called triad. The number of tests where a choice was made by the male is between brackets.

	Tube	**Arena**	**Both**
Individuals			
Females	84	92	76
Males	42	58	38
Tests			
Tested triads	42 (35)	46 (29)	16
Repeated triads	24 (21)	12[a] (5)	22
Total tests	66 (56)	58 (34)	38

[a]Triads tested with a different male.

Statistical analyses

Female tick weight was individually measured twice to the nearest 10^{-2} mg (Mettler Toledo XS205) and the average value was used in the analyses. Analyses were carried out in R 4.1.2 (R Core Team 2020). Preference for each female was binarily coded. Generalized linear models (logistic regression) were used to analyse differences in engorgement weight between chosen and not-chosen females. For every test, male choice was set as response variable while the weight difference between ticks was set as explanatory variable. To avoid pseudoreplication, separate models were calculated for every setup and order of test, i.e. first or second test, unless we specifically tested repeatability. Male mating repeatability was analysed by comparing the proportions of males that chose the same or the opposite female using a two-sample Z-test for equality of proportions with continuity correction, using only males that made a choice in both tests. We analysed repeatability between setups using the binomial exact test instead of the chi-squared test as it is considered to better perform at low sample sizes (McDonald 2009).

Results

When analysing only the triads tested for the first time, chosen females tended to be slightly heavier than non-chosen females both in the horizontal tube (median: 44.87 vs 43.30 mg) and in the arena (median: 47.40 vs 41.02 mg) but weight difference was not a significant predictor of male choice (tube: estimate = 0.00, df = 33, $P = 0.77$; arena: estimate = 0.00, df = 27, $P = 0.27$; Fig. 2). Results were similar when we considered all tests, i.e. including the repeated ones (tube: estimate = 0.00, df = 54, $P = 0.92$; arena: t = 0.00, df = 32, $P = 0.33$).

When testing the repeatability of male choice in the absence of tactile cues (horizontal tube), five males chose the same female (21%), 14 chose the opposite one (58%), and five more males did not make a choice in at least one of the two tests (21%).

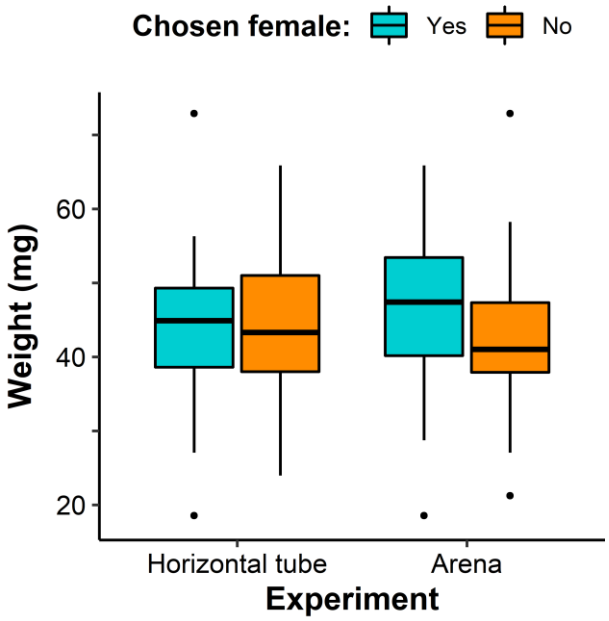

Figure 2. Difference in engorgement weight between chosen (light blue) and not-chosen (orange) adult female ticks in the horizontal tube (N = 35) and arena (N = 29) setup. Engorgement weight median (horizontal line), first and third interquartile range (box limits), minimum and maximum values (vertical lines), and potential outliers (dots) for both groups of females are shown.

Hence, males significantly preferred the opposite female (or the same side of the tube) during the repeatability test (χ^2_1 = 6.737, *P* = 0.009; excluding the males that made only one choice). Repeatability of male mate choice between the tube and arena setup was not significant, as nine males chose the same female, 10 chose the opposite one, and 19 made no choice (χ^2_1 = 0, *P* = 1).

In the horizontal tube, males spent more time (on average 17 vs 6 min) in the arm of the tube corresponding to the female they subsequently chose (Wilcoxon rank sum test: *P* < 0.001) but no significant difference was found between heavier and lighter females (Wilcoxon rank sum test: *P* = 0.10).

Discussion

Our results show that *I. arboricola* males do not show any preference for heavier engorged females although these are expected to produce more offspring (Fracasso et al. 2022a; Ginsberg et al. 2016; Ma et al. 2013; Van Oosten et al. 2016a). This lack of male mate choice for body size occurred both in the absence and presence of tactile cues. Very few observations on mate choice are available for other tick species. For instance, in a population of *Dermacentor andersoni*

polymorphic for body size (bimodal distribution), small males mated more frequently with large females but large males mated equally frequently with large and small mating partners (la Fuente et al. 2005). Although we did not account for male weight, *I. arboricola* males are monomorphic for body size and hence less likely to show differences in preference between bigger and smaller males. In the wild, genotype analyses of *I. ricinus* showed evidence of assortative mating, a potential sign of mate choice although the factors driving it are unclear (Kempf et al. 2014).

A number of non-mutually exclusive hypotheses may explain the absence of male choice. First of all, males may have been unable to correctly assess female size. We do not know to what extent this tick species relies on chemical, tactile or other cues to find and assess potential mating partners. Alternatively, the benefit of choosing between females may be lower than the cost of female assessment. It is worth mentioning that the accuracy of the prediction on the quality of a potential mate plays an important role in determining such costs.

Moreover, engorged ixodid females have little chances to avoid mating due to their impaired mobility. Ixodid females may thus greatly benefit from operating cryptic choice of male sperm after insemination has occurred. Cryptic female choice has crucial evolutionary implications (Firman et al. 2017) and may hamper the evolution of male mate choice. Although cryptic female choice has been extensively documented in arthropods (Peretti and Aisenberg 2015) very little is known about cryptic female choice in ticks. A previous mating experiment with *I. arboricola* showed that there was less mixed paternity than expected by chance based on the overall paternity ratio.

From an evolutionary point of view, preprandial mating may also have hampered the evolution of male mate choice through the spread of the reproductive investment across several mating partners. This would reduce the costs associated to mating with a lower quality mate. Finally, our experimental males were kept in individual vials until testing. Hypothetically, the prolonged lack of females may have induced males to estimate that the risk of not mating was high. Males might thus

have responded to these environmental conditions by reducing their choice behaviour in order to mate as quickly as possible. Plasticity in mate choice has been shown to vary with environmental and social conditions as well as with the chooser's characteristics in many animal species (Ah-King and Gowaty 2016). In particular, both game-theoretical models and experimental evidence in arthropods have shown density-dependent plasticity in mate choice (Fawcett and Johnstone 2003; Kelly 2018; Lehmann 2007). We suggest more studies should assess whether tick mating behaviour is affected by the presence of potential mating partners.

Interestingly, *I. arboricola* males that were tested twice in the same setup (horizontal tube) showed a preference for females that were not previously visited, instead of repeating their choice. One hypothesis is that males may have recognized the previously encountered females and have avoided the one with whom they were previously unsuccessful. This hypothesis assumes that males somewhat remembered their previous mating partners for at least a couple of days. This could be done through chemosensory recognition, for instance. A wide range of arthropods has been shown to use cuticular hydrocarbons as recognition cue during mating and utilize chemosensory self-referencing to identify recent mates thus requiring minimal cognitive abilities (Weddle et al. 2013). Alternatively, males may aim to spread their mating effort over different females, but this seems rather less likely since in this case males would need to remember the previous mating partner but overlook that the mating was unsuccessful. However, our setup cannot rule out confounding factors due to the fact that female positions were not randomized but always alternated between the two ends of the tube. Thus, males may have had a preference for moving towards one specific side of the tube (which always had the same position with respect to the observation room). We believe this hypothesis is unlikely since there was no general preference for one particular side of the tube. Nevertheless, for unknown reasons individual males might have had different preferences. Or alternatively, males may have preferred to move in the same

direction of the tube where they had encountered a female the previous time, i.e. use this as a cue for an environment where females can be found.

Our findings suggest that *I. arboricola* males can exert choice in the absence of tactile cues. Ideal candidates to convey such information are pheromones. Although sex pheromones have not (yet) been identified in ixodid ticks (but see Carr et al. 2016), a large number of volatile and non-volatile pheromones has been shown to be involved in several phases of the life cycle of Ixodidae (Sonenshine 2006; Sonenshine and Roe 2013). We hypothesize that *I. arboricola* males may use pheromones secreted by females to recognize them. The use of sex pheromones had previously been suggested by other studies in *I. ricinus* (Bouman et al. 1999; Bouman et al. 2003; Zemek et al. 2007) although there is no general consensus (Leonovich 2020). Interestingly, when triads tested in the tube were later tested in the arena, males did not show a preference for the opposite female. One explanation could be that the volume of air was much bigger in the arena and pheromone gradients would have been much more diffuse than in a linear setup. Alternatively, the different result could lend support to explanations based on males remembering a specific location (i.e. the arm of the tube) than an individual female. In this case, carrying out additional tests in the absence of the females could help to shed more light on male tick behaviour.

Conclusions

In conclusion, we suggest that *I. arboricola* males do not prefer to mate with heavier and thus more fecund engorged females in the wild, nor did we find evidence for consistent preference for individual females across trials. On the other hand, outcomes suggest that males may recognize individual females. An important implication is that subsequent choice tests separated by only one or two days may not provide independent results, and that possible memory effects for specific locations have to be taken into account. More studies on the role of sexual selection in ticks and other arthropod vectors would not only be beneficial for a better

understanding of their mating mechanisms but also for a better comprehension of the selective pressures acting on these parasites.

Chapter VI

General discussion

and

future perspectives

The study of host-parasite interactions has long interested scientists for its manifold implications ranging from evolutionary ecology to disease dynamics. The current pandemic situation abruptly emphasized to the general public the global consequences that host-parasite relationships can have on countless sectors of human life. Furthermore, parasites are mainly seen as evolutionary and ecological forces rather than as species on their own (Poulin 2007). To change that, this dissertation aimed at investigating the individual variation and evolutionary potential of life-history and success traits in a hematophagous ectoparasite, the tree-hole tick *I. arboricola* as well as to investigate to what extent these traits are influenced by the host. To do so, I investigated a number of traits and success parameters associated with life on host – attachment success, feeding time, engorgement weight, feeding success – and off host – moulting time, moulting success, and overall survival. The findings shown in this dissertation are discussed in the context of host-parasite interactions and coevolution, focusing in particular on hard ticks. Below, I chronologically review the main challenges faced by hard ticks during their life cycle and summarize how the data presented in this thesis improve our understanding of tick evolutionary ecology at each of these time points. Moreover, I point out the main knowledge gaps that may have affected this work and outline promising new research avenues.

Section I: selective pressures in the tick life cycle

Finding a host

In order to engorge ticks need to find and exploit a suitable host. In the case of *I. arboricola*, individuals do not quest actively but rather wait for birds to come and roost or breed in the cavity. This characteristic makes *I. arboricola* heavily dependent on bird roosting and breeding phenology (Heylen et al. 2014c). Although host choice has not been investigated in this dissertation, this phase likely represents a fundamental choice in the tick life cycle. In fact, in **chapter IV** I show that even within host species individuals differ in quality affecting parasite performance. Even though

I. arboricola has very limited mobility it can survive for months or years in cavities thus potentially allowing it to come into contact with several hosts from which to choose. For all experiments presented in this dissertation ticks were not allowed to choose on which host to feed. This difference compared to natural conditions may have reduced the attachment and feeding success of our tick population but it is unlikely to have significantly affected attachment preference on the host body, the predictors of tick success or trait correlations. However, since very little is known on host choice behaviour and its physiological, ecological and evolutionary consequences on tick traits and performance, I argue that this research topic deserves further investigations (see "Section II: perspectives and concluding remarks" below).

On host

Attaching on the right place

On the host ticks need to find a safe place where to exploit host resources for several days. For instance, ticks may completely fail to acquire a sufficient amount of blood if they attach on a highly keratinized region. Alternatively, they could find a suitable area but be groomed away by the host while feeding (Clayton et al. 2005). A variable that can be taken as a proxy for these selective pressures is attachment success. In this dissertation I show that tick attachment success depends on multiple intrinsic and extrinsic tick characteristics. In **chapter II** I report highly consistent attachment preferences between tick species, stages, and individuals irrespective of the wide differences in ecology and host specificity. Ticks were almost exclusively found on the head and neck while they were absent in all other body areas. This is in line with an extensive amount of literature observing the same pattern in several other bird-tick systems (see references in Table 1). By means of four different experiments using *I. arboricola*, *I. ricinus*, and *I. frontalis* feeding on two songbird species I show that the proximate cause of the observed pattern of attachment is not the selective removal of ticks from specific areas of the body from the host but it is

rather due to the active movement of ticks towards the head. When ticks were placed on other body parts they moved to the head if given the opportunity. Interestingly, attachment success was lower if ticks were forced to remain in a body area other than the head even in the absence of host grooming suggesting that ticks refused to attach in these suboptimal conditions. In the study, I hypothesize that this tick behaviour is an adaptation to the selective pressure exerted by host grooming. Ticks thus prefer the bird head and neck as they are much more difficult to reach in the absence of reciprocal grooming from conspecifics, i.e. allo-grooming (Bush and Clayton 2018). In fact, the beak of a conspecific could easily reach body areas such as the head and neck that cannot otherwise be groomed by the host. Experiments on tick attachment preferences between host species with and without allo-grooming would allow to investigate whether the latter factor plays an important selective role. Simple mechanisms may drive ticks towards the head. For instance, the negative geotropism shown by many tick species at the onset of questing (e.g. *I. ricinus*) or after engorgement (e.g. *I. arboricola*) may also help ticks to locate the bird head. Alternatively, ticks may follow a temperature and/or a carbon dioxide gradient.

The experiments and literature evidence reported in **chapter II** strongly suggest that this behaviour is consistent throughout ixodid ticks exploiting songbirds. Interestingly, ticks are more scattered throughout the body of seabirds (Barton et al. 1995; Choe and Kim 1988; Danchin 1992), maybe due to their lower overall grooming efficiency associated to webbed claws. In fact, scratching with the claws and preening with the beak work synergistically to remove ectoparasites (Goodman et al. 2020). The behaviour of *I. ricinus* is also worth noting. In fact, this generalist tick is found attached on different body parts when it exploits reptiles (Bauwens et al. 1983), cattle (L'Hostis et al. 1994), or humans (Wilhelmsson et al. 2013). Assuming that in these species the attachment pattern is also driven by parasite preference, it can be hypothesized that generalist ticks – and possibly other ectoparasites as well – have different attachment preferences based on the host species that they are currently exploiting. This could be an adaptive strategy if the chances of survival between body

areas differ between birds, reptiles, and mammals. To test this hypothesis, the relationship between tick survival in different body areas and attachment preferences across host taxa should be further investigated. A non-mutually alternative hypothesis is that ticks benefit from the micro-environmental conditions found on the head, possibly mediated by morphological characteristics such as feather density (Barton et al. 1995). In this context, it should be noted that the bird head is a highly vascularized area. However, it is currently unknown if the morphological properties of the area where ticks are attached affect their performance. The use of artificial skin as a medium for tick feeding (Krull et al. 2017) might help to address these questions in future studies as it would allow to experimentally manipulate several skin properties (e.g. keratinization, thickness). Lastly, attachment on the head implies that ticks feed in close proximity with the central nervous system of the host, possibly increasing the likelihood of paralysis due to tick feeding. However, it should be noted that only about 8% of ticks cause paralysis (Pienaar et al. 2018).

Besides the site of attachment, other factors may determine whether a tick successfully attaches on the host. The thorough investigation of *I. arboricola* performance and life-history traits reported in **chapter III** shows that a longer moulting time between the nymph and adult stage significantly increased attachment success of adult females. Although my findings do not rule out the presence of different life-history strategies they rather support variation in tick quality although the proximate and ultimate causes underpinning such variation remain unclear. In this dissertation, tick variation and tick quality are different measures. Tick quality is the capacity of an individual to cope with multiple selective pressures. Quality thus has a more extensive meaning respect to fitness, i.e. the reproductive capability of an organism, where the latter is used as a measure of the former. For instance, in my dissertation I show that the ticks with the heaviest weight acquired these resources in less time and even moulted faster thus increasing their fitness. To the best of my knowledge no other studies investigated individual

variation in parasite quality. The correlation between moulting time and tick quality might be underpinned by (unidentified) physiological and/or genetic mechanisms. As a hypothesis, ticks of higher quality may have a set of alleles allowing them to be quicker in converting blood during moulting. The advantage given by this set of alleles may however be linked (by the genetic architecture) with other alleles that reduce the feeding success of adult females.

Interestingly, I could show that part of the variation in attachment success depends on host quality. In particular, the results of **chapter IV** show that attachment success of larvae and nymphs feeding on the same individual host are positively correlated in separate infestations and independent from temporal variation. This suggests that individual host characteristics similarly affected different parasite stages. Additionally, I tried to identify the individual host traits significantly affecting tick attachment success. Although the change in body condition and haematocrit were found to be related with tick attachment in larvae and nymphs respectively, the set of traits and characteristics driving variation in host quality for this parasite trait are far from being fully elucidated. Importantly, I found heritable variation in host quality for tick attachment success, especially for host effects on larvae. This means that hosts can exert long-lasting selective pressures on their parasites thus contributing to shape host-parasite coevolution. The available literature suggests that significant variation in host quality could also be present in other host-parasite systems both within (Devevey and Brisson 2012) and between parasite stages (Payne et al. 2020). Hence, attachment success in other tick species might be similarly regulated. As stated in **chapter IV**, variation in host quality can considerably affect both disease dynamics and host-parasite coevolution. In fact, variation in host characteristics might trigger (counter)adaptations in the parasite with effects on the parasite-transmitted pathogens. For instance, a change in the shape of the beak (e.g. in response to the diet) can affect preening and thus attachment success on the host body. The reduced selection on the parasite may allow for a more even tick attachment distribution on the host body that in turns reduces the probability of co-

feeding transmission of pathogens between ticks. Thus, all other variables remaining equal, the disease spread will be reduced. However, knowledge on the underpinning dynamics is currently scant and deserves further attention.

Measuring attachment success requires counting the number of ticks put on the host and those that effectively attached. This can easily be done for nymphs and adult ticks while counting dozens of unfed larvae is much more time consuming due to their body size more heavily affecting larval energy reserves. For this reason, in this thesis the number of larvae was estimated after quickly counting them twice. This led to a sufficiently good approximation (about ±5 individuals) with a reduced impact on larvae. Hence, measurement error for attachment success could have been slightly higher for larvae compared to the other stages.

How long to feed?

After attachment, ticks start feeding, a process lasting several days. Theoretically, longer feeding times could allow to acquire more resources from the host although having an extended risk of being groomed. Moreover, host individual characteristics may influence tick feeding time by facilitating or hindering the exploitation. I investigated these hypotheses by measuring feeding time, feeding success, and engorgement weight (see below for the latter). The findings I report in **chapter III** and **IV** strongly suggest that feeding time is affected by tick quality in interaction with host quality. Interestingly, when compared with other parasite traits such as engorgement weight or moulting time hosts seems to more strongly affect feeding time. Also, my experimental work shows that feeding time is negatively correlated with engorgement weight and moulting success while it positively correlated with moulting time in both the larva and nymph stage (**chapter III**). Moreover, the number of hatched eggs decreased with feeding time. Hence, longer feeding times may indicate that ticks are inefficiently exploiting their host or that these ticks are of low quality. Feeding time is also the trait with the lowest

evolutionary potential in ticks. This trait was also the most affected by host heritable characteristics, in particular in larvae.

In ectoparasites whose attachment last for days such as ticks, feeding and attachment success are intrinsically correlated but show substantial differences. In fact, after attachment parasites can differentially be affected by host individual behaviour (e.g. grooming) and its immune system (not in the host-parasite interaction studied here), thus reducing parasite feeding success. The site of attachment itself might also play a role by offering more or less protection to host behavioural and physiological defenses or, most probably, by providing a different amount of resources (as I hypothesize in **chapter II**). The close relationship between attachment and feeding success is also supported by the common influence of moulting time on both performance parameters (**chapter III**). Specifically, in adult females a longer moulting time from the nymph to the adult stage was positively correlated with a higher feeding success, similarly to what was observed for attachment success. As for other traits, in **chapter IV** I show that feeding success was significantly affected by host characteristics with larvae being less successful on female hosts. The substantial effects of host individual characteristics on tick feeding success are also supported by previous studies on the congeneric *I. ricinus* tick in which considerable variation between hosts in the tick's feeding success has been observed (Heylen et al. 2010; Heylen et al. 2015). Lastly, I observed sex-related differences in parasite load similarly to what is observed in several parasite-host systems in the wild (Roberts et al. 2004; Ruiz-Fons et al. 2013; Tschirren et al. 2003), including ticks (Heylen et al. 2013a).

How much to engorge?

During feeding, ticks increase the amount of stored blood thus increasing their weight. On the one side, it can be hypothesized that tick engorgement weight is directly correlated with more available resources and higher fitness, and this may be true at every stage. On the other side, a lower amount of blood ingested may reduce

feeding time and thus exposure to host grooming. Moreover, ticks may be able to moult faster and be ready for a new feeding event sooner as they need to digest less blood. It can thus be speculated that ticks trade off engorgement weight with other requirements.

However, the results reported in **chapter III** strongly suggest that ticks try to maximize the amount of resources exploited and that engorgement weight is the best proxy of tick individual quality for several reasons. First, engorgement weight is positively correlated with moulting success and negatively correlated with moulting and feeding time in both larvae and nymphs. Second, the positive correlation in engorgement weight across life stages and the low estimated heritability of host quality for this tick trait suggest that host traits only marginally affected the parasite capacity to engorge. Third, in adult females I show that engorgement weight is positively correlated with the number of offspring produced (**chapter III**). However, it should be noted that a higher engorgement weight does not necessarily translate in a higher amount of ingested blood as my studies did not correct for differences in the weight of unfed ticks. Nevertheless, at every feeding event *I. arboricola* grows in size more than an order of magnitude (**chapter III**) likely overshadowing any initial weight difference between unfed individuals.

Although host characteristics had an overall low effect on engorgement weight, higher haematocrit levels increased engorgement weight. Further studies should corroborate this result and investigate which blood components drive such relationship. In the tick, engorgement weight is moderately heritable and evolvable. Hence, it could be expected that the amount of genetic variation for this trait will be rapidly depleted unless the continuous interaction and coevolution with the host favours the maintenance of a high level of genetic variability (Ebert 2008).

Additional variables such as the tick weight before engorgement and the net weight of blood ingested after correction for tick body size could add further insights on the life history and fitness trade-offs of this species. For instance, if engorgement weight is directly correlated with tick body size my findings would suggest that bigger

ticks have a fitness advantage over smaller ones. This hypothesis clearly deserves further attention. However, in this dissertation measures of body size were not taken for two main methodological reasons. First, a scale with extreme accuracy and sensitivity is needed to weigh unfed larvae and accounting for measurement error can become very challenging. Asides from this, handling unfed larvae individually is impractical. Second, weighing ticks individually is a very time-consuming activity and in the best-case scenario only half of the ticks weighed and put on birds are recovered and can be used in the analyses.

Off host

How fast to moult?

The resources acquired during engorgement permit to the parasite to moult to the next stage. This process is called ecdysis (moulting) and allows the shedding of the outer integument (de Oliveira et al. 2019). Moulting represents a fundamental phase in the life history of an animal due to the associated changes in genetic expression, morphology and physiology (Sonenshine and Roe 2013). In arthropods, it is composed of a highly conserved series of mechanisms regulated by ecdysteroid hormones and neuropeptides (de Oliveira et al. 2019; Sonenshine and Roe 2013). Moulting quickly would allow ticks to accelerate their life cycle by potentially feeding on more hosts in the same season (Heylen and Matthysen 2011a). However, this advantage may be traded-off with a lower quality of the entire process in a sort of "quick-and-dirty" strategy.

The findings reported in this dissertation indeed show that moulting time is very relevant for tick performance and life history. First, moulting time was positively correlated with attachment and feeding success in adult females. Second, in both larvae and nymphs moulting time showed a negative phenotypic and genetic correlation with engorgement weight and a positive phenotypic correlation with feeding time. Third, differences in the length of moulting time were found between sexes for both larvae and nymphs. In the latter case, it can be hypothesized that male

nymphs take shorter to moult as they have less cuticle to produce given they do not feed in the adult stage. A faster moulting may also permit males to win the competition for mating partners and thus be subjected to sexual selection. Instead, sex differences between engorged larvae are more intriguing as both sexes at the nymph stage are morphologically identical, engorge similarly and have the same necessities. However, there is no hypothesis to explain this result to date. Finally, moulting time showed substantial evolutionary potential across all stages that was mirrored by an overall low effect of host quality for moulting time.

It is worth noting that not all ticks manage to moult to the next life stage. I decided to take into account this variable by measuring tick moulting success. Overall, moulting success was higher for nymphs (above 90%) compared to larvae (range 66-86%). Moulting success is known to be correlated with environmental conditions such as temperature and relative humidity (Ogden et al. 2004; Sonenshine and Roe 2013). The species identity and resistance of the host can also considerably influence moulting success (Sonenshine and Roe 2013), further highlighting the importance of host choice. This performance parameter was positively influenced by short feeding times and high engorgement weights. Interestingly, heritability of host quality for moulting success was substantial for both larvae and nymphs. In other words, individual host characteristics affecting moulting success of both immature stages showed a substantial heritability. Indeed, when investigating the host traits associated with tick performance (**chapter IV**) I found that host haematocrit positively influenced larval moulting success while host age affected nymph moulting success. I hypothesize that this effect is mediated by differences in the digestion time of the blood belonging to different hosts, likely due to differences in concentration of unidentified molecules in the bloodstream. Experimental manipulations of the blood components may help to shed light on these mediators. Finally, it is important to mention that at least some tick species seem to go through a post-moulting phase during which they are not yet ready to feed successfully (Jones et al. 1988). However, further studies are needed to better understand this phase.

Maximizing offspring number

The final challenge that an organism needs to overcome is to maximize its contribution to the future generation. To investigate that I studied how sexual selection can contribute to shape individual fitness and what predicts the number of offspring produced. Despite the positive correlation between engorgement weight and clutch size, adult males do not show any preference for heavier females (**chapter V**). I believe this result is somewhat surprising giving that *I. arboricola* shows many life history and ecological characteristics that should favour the evolution of male mate choice. For instance, males show mate guarding that can last for several days (Van Oosten et al. 2016a) and adult females are likely to detach from nestlings almost simultaneously (Heylen and Matthysen 2011b) since they prefer to attach on chicks of a very specific age (Heylen et al. 2012). In order to investigate how tick traits correlate with the number of offspring produced I then moved on to study the number of hatched eggs and the success in oviposition. Ticks are semelparous species that reproduce at the very end of their life cycle. Hence, they are expected to invest all their resources in this single reproductive event.

Chemical communication plays a crucial role in the life cycle of a tick regulating a wide range or processes from host finding to courtship and oviposition (Sonenshine 2004; Sonenshine and Roe 2013). For instance, one of the main cues used to locate a host is the detection of carbon dioxide by means of the Haller's organ (Carr and Salgado 2019). Also, ticks secrete chemical molecules in the environment (semiochemicals) to allow communication with conspecifics (e.g. aggregation pheromones) or defend themselves against predators (Yoder and Domingus 2003). Tick reproduction is also regulated by sex pheromones. Several pheromones have been identified in metastriate ticks while the genus *Ixodes* received less attention (Sonenshine 2004). Understanding the functioning of such molecules might allow us to enhance tick control, in particular when these semiochemicals are associated to acaricide molecules (e.g. "attract and kill" devices).

As I report in **chapter IV**, both a higher engorgement weight and a lower feeding time increase the number of larvae sired by a female. While the correlation between engorgement weight and clutch size is rather common in ticks (Chen et al. 2009; Ginsberg et al. 2016; Gray 1981; Ma et al. 2013), to the best of my knowledge the negative correlation between feeding time and number of hatched eggs has never been previously reported. As regards egg-laying success, this was lower for females that attached at the second infestation, possibly suggesting a relationship with tick quality. One hypothesis is that females that attached at the second infestation attempt were in suboptimal conditions and this was then reflected on their probability of egg laying.

Interestingly, in both Van Oosten et al. (2016a) and in this thesis egg-laying success was approximately 80-85% (sample size bigger than 280 adult females). Future studies should investigate egg-laying success in the wild as no information is available to date and it is not possible to understand if lab conditions decreased or increased egg-laying success compared to the wild.

Considerations on the entire life cycle

Overall, comprehending the processes that determine parasite survival within every life stage in the wild is a daunting challenge. Survival is in fact the outcome of all the selective forces mentioned above acting at every phase of the parasite life cycle. **Chapter III** and **IV** thoroughly investigated them from the perspective of the parasite and host respectively. None of the life-history traits under study (feeding time, engorgement weight and moulting time) predicted survival in nymphs and adult females. Moreover, heritability of host quality for survival in larvae was substantial further indicating that individual host variation affects parasite fitness. Hence, variation in host quality seems to have a bigger effect on tick survival respect to the recent parasite life history.

Interestingly, the time elapsed between consecutive feeding events (fasting time) affected survival negatively in nymphs and positively in adult females. Fasting

time is rarely considered in studies on ectoparasites as the relationship between fasting time and parasite performance is generally neglected. In **chapter III** and **IV** fasting time encompasses a wide time window (5 – 155 days). Although freshly hatched or moulted ticks might have lower feeding success (Jones et al. 1988), the vast majority of ticks used in this dissertation was put on birds several weeks after moulting (median days elapsed after hatching or moulting: larvae = 102, nymphs = 74, adult females = 57). Hence, it is unlikely that incomplete tick development may have significantly affected the results presented here. Furthermore, a negative correlation between tick age and feeding success was also found for larvae of Rocky Mountain wood ticks (Jones et al. 2015). Somewhat surprisingly, I instead show that fasting time also had several other significant effects on tick performance and life history traits. In fact, fasting time also affected feeding and moulting success as well as feeding time. Hence, given its seemingly pervasive effects on parasite traits I suggest that fasting time should be accounted for in future experiments on ectoparasite traits to reduce its confounding effects. Differences in the parasite energy reserves and in their management may underlie these effects.

When considering the tick life cycle as a whole, the findings reported in this dissertation suggest that, with the exception of tick preference for attachment sites (**chapter II**), there is considerable intraspecific variation in tick quality for which engorgement weight seems to be a good proxy. Similar studies carried out in a range of other ectoparasite species would allow us to understand how much my results can be generalized given the wide diversity of host-parasite systems. Moreover, I show that individual variation in host characteristics significantly affects parasite traits. Importantly, I could show that at least some of these host selective pressures are heritable and can thus be exerted on evolutionary timescales. In particular, attachment and feeding success seem to be the traits more heavily influenced by intraspecific host variation while for other parasite traits host effects were low.

In this thesis, I avoided to make predictions on future evolutionary trends in the *P. major-I. arboricola* interaction as this was out of the aims and scopes of the

thesis. Moreover, although the comparison between the predicted and the observed evolutionary change could considerably enhance our understanding of evolutionary and ecological processes (Nosil et al. 2020), accurate predictions for *I. arboricola* are severely constrained by data limitations. Nevertheless, it can be hypothesized that the results shown here are the outcome of a long coevolutionary history in the parasite-host system under study. In this case, we should not expect the rapid evolution of adaptations in either the parasite or the host provided that extrinsic (e.g. environmental) and intrinsic (e.g. physiological) conditions remain stable. However, my findings (**chapter III** and **IV**) clearly show that *I. arboricola* traits have the potential to evolve and that great tit characteristics do exert selective pressures on ticks. Hence, this host-parasite system should not be considered static. From a theoretical viewpoint, it would be extremely interesting to individually follow a tick in a naïve tick-host system (ideally for several years) in order to investigate how and to what extent the parasite and the host adapt to each other throughout generations and whether there is a cost associated with host specialization (Draghi 2021). Furthermore, it is unclear if there is a general pattern across parasite-host systems on how, and to what extent, hosts affect parasites.

From the host perspective, this dissertation shows consistent variation between individuals in tick load (**chapter IV**) but did not investigate the evolutionary potential of great tit traits in response to *I. arboricola* selective pressures nor host inter-individual variation in tick tolerance or resistance. Hence, it is unclear if hosts are currently coevolving with the parasite. However, given that *I. arboricola* seems to have only a (very) minor effect on great tit fitness, the selective pressures driving the evolution of host adaptations are expected to be similarly low. Nevertheless, it should not be neglected that hosts may evolve adaptations to other ectoparasites or to selective pressures unrelated to parasite defence but that also prove to be effective against ectoparasites such as *I. arboricola*.

The follow-up of individuals throughout multiple life stages allows to address a long-standing question in evolutionary biology, namely how much (evolutionary)

independence there is between traits expressed in different life stages (Collet and Fellous 2019). In principle, traits expressed at different life stages could be completely independent from each other as they are affected by different selective pressures. However, complete genetic independence is believed to be rare due to the very same architecture of the genome (e.g. linkage disequilibrium). In *I. arboricola*, every stage seems to be at least partially affected by different selective pressures despite the common environment and similar feeding strategies between stages. Hence, I hypothesize that parasites whose stages radically differ in morphology and feed on different host species and/or environments might show an even bigger separation in traits and selective pressures between stages. Comparative studies between multi-stage parasites could help to address this question.

Ixodes arboricola larvae seem to be the life stage most sensitive to environmental (including host) conditions. This may be due to their highest surface-to-volume ratio compared to the other stages. Also, larvae are the stage with the lowest amount of lipids, the main energy reserve of ticks, thus giving them less opportunities to compensate for dehydration (Rosendale et al. 2017). Given that these two characteristics are shared across tick species, I hypothesize that larvae are the most sensitive stage across tick species. This can have important evolutionary and ecological implications as larvae are the most abundant stage in the wild and, at least in *I. arboricola*, they are also fundamental for the dispersal of the species (Van Oosten et al. 2014a). However, it should be noted that larvae are the stage that is more easily affected by measurement errors due to their small size, in particular for engorgement weight. Nevertheless, in all the experiments reported here great care was taken to minimize the relative measurement error for this stage. For instance, engorgement weight was always measured twice for every tick individual and the average weight was considered for the statistical analyses (all stages).

Section II: perspectives and concluding remarks

Disease ecology

Ixodes arboricola seems not to have measurable negative effects on haematocrit, inflammation, body size, and body condition of great tit nestlings (Heylen and Matthysen 2011a; Van Oosten et al. 2014b). The direct burden caused by tree-hole ticks may be compensated by the hosts or simply be very low. From the tick perspective, consecutive infestations with a congeneric species of *I. arboricola*, namely *I. ricinus*, in great and blue tits did not affect tick attachment success, feeding time, engorgement weight, moulting time, and moulting success indicating a lack of acquired resistance in the hosts (Heylen et al. 2010). This happened despite an increase in the specific antibodies against *I. ricinus* salivary proteins over consecutive infestations (Heylen et al. 2021). It is likely that a similar immune response (and lack of acquired resistance) also occurs in the *P. major-I. arboricola* system although *I. ricinus* and *I. arboricola* belong to different phylogenetic clades (Charrier et al. 2019; Heylen et al. 2014a). Hence, as also stated in **chapter IV**, the increased bird immune response across consecutive infestations is seemingly not affecting *I. arboricola* fitness.

Besides the direct effects that ticks have on their hosts, a wide range of pathogens can also be transmitted (Boulanger et al. 2019; Cutler et al. 2021; Dantas-Torres et al. 2012). In this respect, *I. arboricola* seems not to be a competent vector for the transmission of *Borrelia* spp. as the spirochete is transmitted to the tick from an infected host but it cannot be transferred to the next host (Heylen et al. 2014b). However, it may still be a competent vector for the transmission of Rickettsiae (Palomar et al. 2015; Špitalská et al. 2011) transmitting pathogens to non-avian hosts through co-feeding with *I. ricinus* (Kocianová et al. 2017).

Parasite traits are closely related to disease transmission. For example, both a consistent attachment preference for a specific body area (**chapter II**) and longer feeding times (**chapter III**) can enhance disease transmission as they give higher chances to pathogens to spread across ticks during co-feeding (Randolph 2011;

Randolph et al. 1996). Moulting time is also relevant as it determines the seasonal overlap between feeding tick species and stages. Lastly, the dispersal and success of tick-borne pathogens is intrinsically linked to the survival of its vector. In hard ticks, if survival throughout stages is high then pathogens have higher chances to be transmitted to new hosts provided that tick abundance remains constant. Similarly, vertically transmitted pathogens will benefit from more fertile females (**chapter III** and **V**). These examples point out the importance of further investigating the relationship between parasite traits and disease transmission, especially given to the paucity of empirical data.

Ticks as study systems

Besides ticks, few other taxa of parasites allow such a detailed investigation of their life history, phenotypic and genetic trait correlations, trait evolutionary potential, and behavioural preferences. In fact, parasites living inside the host (endoparasites) and microparasites are hard or impossible to follow individually. Several characteristics can be listed that make ticks good study species for the comprehension of host-parasite interactions.

First, ticks are easily stored in laboratory conditions for months with minimal requirements and they can be fed in semi-natural conditions. Second, ticks feed only a few times in their life cycle and cannot switch host before feeding is completed thus reducing the complexity of the system. Third, their size allows to mark them individually while still being able to store many individuals in a small space. Fourth, ticks have multiple life stages that are sufficiently large to be individually studied. Fifth, future studies can further delve in the host-vector-pathogen interaction. These studies will allow us to better comprehend how the selective pressures acting on different biological entities (e.g. host, vector, pathogen) interact antagonistically or synergically between each other (de la Fuente et al. 2016; Hovius et al. 2007). Furthermore, the heritable and non-heritable effects given by every entity of the interaction can be estimated. Sixth, hosts can be infested in a controlled way and

ticks can easily be recovered afterwards (McCoy et al. 2002; Van Oosten et al. 2014b, chapter II). Seventh, ticks reproduce sexually thus allowing to include the effects of sexual selection to the study of the parasite evolutionary ecology.

Some of these desirable characteristics are shared with other acari, mosquitoes, and lice. The latter in particular have provided a significant contribution to the comprehension of host-parasite interactions and coevolution (Clayton et al. 2015; Poulin 2007; Villa et al. 2019). Interestingly, lice spend most of their life cycle on the host while ticks and mosquitoes live mostly off the host. This difference provides an excellent but still overlooked opportunity to study how different ecologies may affect parasite adaptions and survival. However, ticks also have drawbacks as study systems. For instance, they have slow life cycles that hinder studies of experimental evolution (e.g. Villa et al. 2019) and the creation of genetic lines with specific characteristics (e.g. Venken et al. 2011). Also, introducing pathogens and other experimental molecules (e.g. markers) through the diet is currently nearly impossible.

The microbiome

Several elements could modulate tick behavioural preferences and its life history. In this respect, recent findings pointed out the importance of the parasite's internal environment, and in particular its microbiota (Benelli 2020; Takken and Verhulst 2013). An increasing amount of evidence shows that symbiont and commensal microorganisms affect tick physiology and behaviour through multiple pathways thus increasing their own transmission probability (Benelli 2020). Furthermore, many of the microorganisms that have been shown to manipulate their vector are also pathogenic for humans and farm animals thus making their study even more crucial.

In ticks, most studies have focused on *Borrelia*, the causative agent of Lyme disease. For instance, it has been shown that *Borrelia* upregulates the production of a tick histamine release factor in *Ixodes scapularis* likely facilitating tick engorgement

and pathogen transmission (Dai et al. 2010). As a general trend, symbionts and commensals can largely contribute to increase tick fitness in a win-win strategy with their vector (Benelli 2020). Given the available evidence, I think it is of fundamental importance to better understand the effect that the microbiome has on ticks and other parasites. Furthermore, tick co-infection with multiple pathogens and symbionts seems to be the rule rather than the exception (Moutailler et al. 2016). Even though these microorganisms interact between each other with important implications for the vector and the host (Bonnet et al. 2017; Cutler et al. 2021) these interactions are currently poorly understood (Cutler et al. 2021).

Host choice and its plasticity

The parasite microbiota may also partially affect (plasticity in) host choice, another key element in the parasite-host interaction. In fact, due to the inter- and intraspecific variation in host quality that I observed, it can be speculated that ticks and other ectoparasites assess the characteristics of potential hosts before they commence feeding. Since parasite requirements and selective pressures change between life stages and with changing internal (e.g. microbiome) and outside conditions, parasites might also adjust their choice in order to maximize their fitness throughout the life cycle. For instance, different stages may prefer hosts with different characteristics even within the same host species (host choice plasticity). For instance, plasticity in host choice may explain the different host distribution of *I. ricinus* between stages (Cull et al. 2018; Matuschka et al. 1991).

Increasing evidence in both lab and wild parasite populations suggests plasticity in host choice in ectoparasites. For instance, it has been shown that host morphology and physiology influence host choice (Caro et al. 2014; Christe et al. 2007) as well as the parasite's previous feeding experience and its microbiome (Benelli 2020; Takken and Verhulst 2013). Host choice plasticity could entail far-reaching evolutionary and ecological implications for both the host and the parasite such as defining under which conditions host species and individuals face a higher

parasite burden. However, it is unknown to what extent host choice affects parasite fitness. Furthermore, the drivers of host choice are to be further elucidated.

Morphological differences

Once on the host, tick morphological characteristics come into play. Attachment success between host species and individuals could in fact be influenced by tick morphological characteristics, in particular with respect to mouthparts. Ticks use their chelicerae and hypostome to cut through the host skin and attach (Richter et al. 2013; Vancová et al. 2020). As mentioned in the general introduction, the hypostome is provided with spine-like denticles that are functional to attach to the host skin. Hence, individual differences in hypostome dentition may affect the tick capability to attach to different host species and/or individuals, in particular if hosts differ in skin thickness or keratinization. Interestingly, extensive variation in the number and distribution of the hypostome denticles has been reported for both *I. arboricola* nymphs and adult females (Haarløv 1962). Although the latter author does not report data for larvae a similar variability can be hypothesized also for this stage. Hence, attachment success in **chapter II**, **III**, and **IV** may have been affected by the variability in hypostome shape between individuals. However, it should be emphasized that no other study further investigated Haarløv's observations. To further investigate this potential source of variation I devised a protocol where the hypostome of every tick could be thoroughly measured from the tick exuvia after reconstruction of the hypostome shape from multiple microscope images (Fig. 1). In fact, the exuvia maintains the imprint of the mouthparts of the stage from which the tick just moulted. During my multi-generational follow-up (**chapter III** and **IV**) I collected and stored more

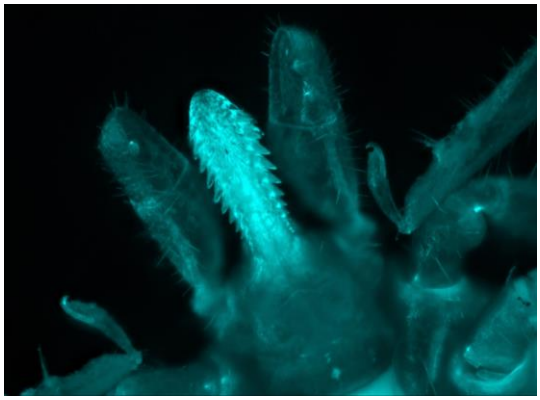

Figure 1. Detail of the mouthparts on an *I. arboricola* adult female. Ventral view. Photo: G. Fracasso.

than 2500 exuviae from the larva-to-nymph and nymph-to-adult ecdysis. To present, preliminary analyses on the samples are being carried out to better investigate Haarløv's observations.

Host individual characteristics

From the host point of view, it has been shown that information on host quality can be transmitted to ticks by odour cues (Dallas and Foré 2013) as chemical volatiles allow to vehiculate information prior to the physical contact with the parasite. Although ticks are known to heavily rely on chemical signals for host finding and reproduction (Sonenshine and Roe 2013) the role of odour cues during host assessment has been mostly neglected (but see Bunnell et al. 2011; Dallas and Foré 2013). Several other characteristics might affect parasite success once physical contact has been established. For instance, attachment and feeding success in ticks and other ectoparasites may be influenced by host integumentary properties such as for instance skin thickness, feather/fur density and length, or the level of keratinization and vascularization (Caro et al. 2014; Marufu et al. 2011). Thus, hosts with a weaker integumentary layout might be more sensitive to parasites thus partially explaining individual differences in host quality. Nevertheless, this hypothesis has been poorly investigated with the few available studies only focusing on tick-cattle systems (Marufu et al. 2013; Marufu et al. 2011).

Final considerations

On a more general level, my findings show that the study of host-parasite interactions and coevolution can greatly benefit from investigating parasites at the individual level. In fact, my study design allowed me to detect the traits predicting parasite success, their evolutionary potential as well as the inter- and intra-stage correlations between traits. Population-level approaches do not allow such detailed investigations and have fundamental pitfalls despite being methodologically simpler (Rueffler et al. 2006). First, parameters that appear as independent at the population

level might not be independent at the individual level where they are generated (Simpson's paradox). For instance, engorgement weight at every stage is generally considered independent from the previous feeding event. However, I here show that this is not the case once individuals are followed throughout their life cycle. Similarly, patterns of covariation between traits might not be conserved at the population level. Furthermore, parameters such as between-stage correlations, alternative life-history strategies, and life-time fitness measures can only be seen at the level of the individual (Clutton-Brock and Sheldon 2010) and many questions in evolutionary ecology can only be answered with individual-level (and possibly long-term) studies (Clutton-Brock and Sheldon 2010; DeAngelis and Grimm 2014; Rueffler et al. 2006). Importantly, individual-based studies would allow to tackle a long-standing hypothesis in evolutionary biology, i.e. that parasite traits are shaped at least in part by similar life-history constraints and trade-offs (within and across stages) as in non-parasitic systems (Poulin 2007) in addition to the constraints and trade-offs related to the interaction with the host.

This dissertation (in particular **chapter III**) demonstrates that parasites can and should be studied individually whenever possible as additional fundamental questions can be addressed. Additionally, this methodological approach would permit the access to statistical methods that are presently confined to non-parasitic species. Indeed, my methodological approach allowed me to use animal models in both the parasite and the host thus allowing to address new questions such as the heritability of traits (Wilson et al. 2010). Because of the wide credible intervals I refrained from quantitative comparisons of heritability estimates, and instead focused on the evidence for presence or absence. However, it should be noted that even low heritability estimates (<10%) may be due to the high residual (environmental) variance rather than due to the low additive genetic variance and thus their evolutionary potential should not be underestimated (Hoffmann et al. 2016; Merilä and Sheldon 2000). In any case, a quantitative discussion of heritability with emphasis to point estimates should be carried out whenever results allow so.

A logical next step is then to take into account both pedigrees at the same time by means of multiple-matrix animal models (Thomson et al. 2018). This statistical method allows to disentangle the relative roles of genetic and non-genetic phenotypic variance by considering the other entity of the interaction (either the host or the parasite) as an autocorrelated environmental source of variation. The methods used in this dissertation coupled with this kind of statistical analysis would allow to fully account for the genetic effects that the host has on the parasite and vice versa as well as to considerably extend the investigation of G × G interactions to many more study systems and natural conditions including (but not limited to) ticks. These data would allow us to shed more light on the general ecological and evolutionary patterns underpinning host-parasite interactions. Furthermore, they will help us to better understand the role of phylogeny and host specificity in shaping the life history of parasites. Also, the versatility of this method permits to account for other sources of spatial and temporal autocorrelations when estimating the additive genetic variance such as epigenetics and cultural inheritance effects (Thomson et al. 2018).

The metabolism of most ectoparasite species is directly correlated with environmental temperature (Poulin 2007; Sonenshine and Roe 2013). In the current context of climate change it is paramount to understand how variation in environmental conditions will affect parasite distribution, life history, and the spread of vector-borne diseases (Aleuy and Kutz 2020; Semenza and Suk 2017). The new climatic conditions might affect tick distributions as well as tick-borne diseases posing new medical and veterinary threats (Gilbert 2021; Gray et al. 2009). However, our knowledge on the direction of these complex phenomena is still very scant (Gray et al. 2009; Randolph 2010). Parasites such as ticks can be individually reared and fed in semi-natural conditions. This allows us to experimentally alter the environmental conditions and thus investigate their effects on parasite traits and performance.

In conclusion, the findings reported in this dissertation show that the study of individual trait variation in ectoparasites provide essential insights on parasite

performance, life history, behavioural preferences, and trait evolutionary potential as well as on the relative contribution that hosts and parasites give to parasite-host interactions. I believe my approach can be similarly used in several other host-parasite systems in order to identify general rules underlying host-parasite interactions. A plethora of research questions awaits to be addressed such as the processes driving host choice and its plasticity, the role of parasite microbiome, and the further investigation of the traits underlying variation in host quality. Exciting new discoveries and compelling questions await us in the near future.

APPENDIX

Supplementary information chapter II

Table S1. Ticks found attached to the head respect to treatment.

Test nr	Experiment	Head	Belly	Back
	No grooming restrictions			
1	Exp. 1, *I. arboricola* larvae	72/120	–	–
2		69/120	–	–
3		94/120	–	–
4		78/120	–	–
5		81/120	–	–
6		–	61/120	–
7		–	63/120	–
8		–	115/120	–
9		–	56/120	–
10		–	74/120	–
11		–	–	78/120
12		–	–	156/120
13		–	–	77/120
14		–	–	126/120
15		–	–	105/120
16	Exp. 1, *I. arboricola* nymphs	6/15	–	–
17		13/15	–	–
18		12/15	–	–
19		10/15	–	–
20		14/15	–	–
21		9/15	–	–
22		12/15	–	–
23		12/15	–	–
24		12/15	–	–
25		8/15	–	–
26		8/15	–	–
27		11/15	–	–
28		6/15	–	–
29		14/15	–	–
30		7/15	–	–
31		12/15	–	–
32		11/15	–	–
33		7/15	–	–
34		6/15	–	–
35		11/15	–	–
36		–	14/15	–
37		–	13/15	–
38		–	3/15	–
39		–	12/15	–

40		–	–	6/15
41		–	–	13/15
42		–	–	10/15
43		–	–	5/15
44	Exp. 3, *I. frontalis* larvae*	63/40	–	
45		8/12	–	–
46	Exp. 4, *I. arboricola*	8/12	–	–
47	nymphs	11/12	–	–
48		6/12	–	–
	With grooming restrictions			
49		12/15	–	–
50		13/15	–	–
51		15/15	–	–
52		11/15	–	–
53		6/15	–	–
54	Exp. 2, *I. ricinus* nymphs	13/15	–	–
55		–	0/15	–
56		–	4/15	–
57		–	1/15	–
58		–	–	0/15
59		–	–	0/15
60		–	–	1/15
61		50/80	–	–
62	Exp. 2, *I. frontalis* larvae	–	1/80	–
63		–	–	12/80
64		14/10	–	
65		18/10	–	
66		9/10	–	
67		16/10	–	
68		14/10	–	
69	Exp. 2, *I. arboricola*	9/10	–	
70	nymphs*	13/10	–	
71		13/10	–	
72		15/10	–	
73		12/10	–	
74		10/10	–	
75		9/10	–	
76		8/12	–	–
77		10/12	–	–
78		–	1/12	–
79	Exp. 4, *I. arboricola*	–	1/12	–
80	nymphs	–	4/12	–
81		–	–	4/12
82		–	–	8/12
83		–	–	7/12

*Bird(s) infested on multiple body parts. The number of ticks found on the head is compared to ticks put on the head.

Supplementary information chapter III

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1. Supplementary methods

1.1. Tick marking and identification

Hard ticks are difficult to mark across stages due to their small size and to the extreme swelling of their body during engorgement. Furthermore, any marking that only affects the tick exoskeleton will be severely modified by the swelling itself.
To overcome these issues, *Ixodes arboricola* was marked by clipping only part of a single limb (four pair of limbs in total). With the exception of the first pair holding the Haller's organ, one limb of the remaining three pairs was cut either at the end or in the middle of the limb. Hence, 12 possible combinations could be used for every bird. Ticks were kept in humid conditions in the short time between clipping and bird infestation to limit water loss. For the clipping we temporarily held ticks in place by gently squeezing them between a hard surface and a transparent plastic film for food packaging. Clipping was done by cutting with a scalpel through the transparent film under a stereomicroscope (for nymphs in the lab) or using a head-mounted magnifying glass (for adult females in the field). Recovered ticks from every nest box (in the lab or in the wild) were temporarily put in a single vial and always kept separate from ticks collected from other nest boxes. Ticks that did not manage to engorge were very rarely found and were removed. We believe that most of them were in fact killed or eaten by the host, or died of starvation or other causes such as due to fungal infection; in the nest boxes, ticks may have moved away without engorging although this is unlikely. Ticks were identified immediately after recovery. The nest box number as well as the limb pair (i.e. II, III, IV), side (left or right) and position of the clipped part (end or middle) were used for identification. Almost all ticks could be unequivocally identified while the few ticks with uncertain identity were removed from the study. Trials before the study and post hoc analyses showed no difference in survival between ticks clipped at the end or at the middle of the limb.

1.2. Cage cleanup

In the lab, cages were thoroughly cleaned with soap and water at the end of every batch. The inner walls of nest boxes were treated with hot air (hot air gun) to kill any unfed ticks that may have remained hidden inside the nest box. Trenches of water surrounding the cages were emptied and cleaned.
In the wild, the old nest material was thrown away at the end of every breeding season.

2. Descriptive data

Table S1. Number of clutches, engorged ticks, feeding and moulting success, and number of infested hosts for the two consecutive tick generations (F1, F2) and their founders (F0). Round brackets in feeding success (females) refer to the outcome of the first infestation only while round brackets in the number of infested hosts refer to the number of nests used.

	F0	F1	F2	Total
Engorged ticks				
Larvae	–	1826	1636	3462
Nymphs	–	332	329	661
Adult females	54	63	65	182
Feeding success				
Larvae	–	69%	72%	70%
Nymphs	–	58%	57%	57%
Adult females	–	42% (28%)	42% (34%)	42% (31%)
Moulting success				
Larvae	–	66%	86%	75%
Nymphs	–	93%	91%	92%
Clutches laid	51	54	55	160
Infested hosts (nests)				
Larvae	–	66	61	127
Nymphs	–	59	71	130
Adult females	(16)[a]	72 (36)	92 (46)	≥ 218 (98)

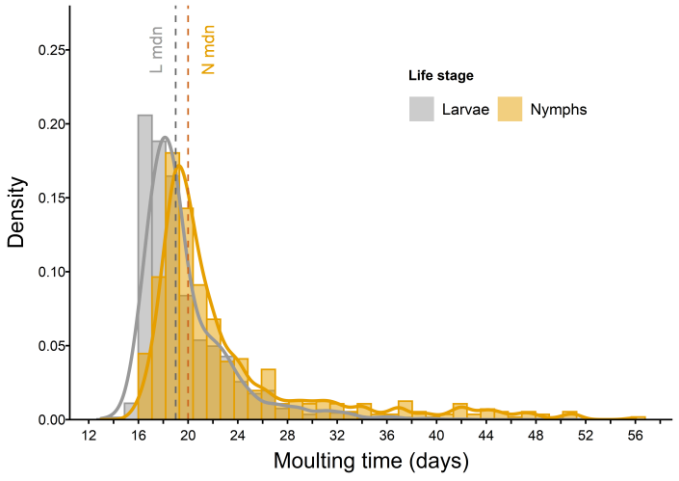
[a]Ticks could have fed on one or more nestlings in the nest box.

Table S2. Mean, standard deviation (±SD), median, and range for feeding time, engorgement weight, moulting time, and number of hatched eggs of all engorged *Ixodes arboricola* collected. Mean (±SD) for moulting time was calculated only on ticks checked every day. Fasting time range (days): 5 – 155 larvae, 42 – 129 nymphs, 60 – 105 adult females.

	Mean (±SD)			Median	Range
	All ticks	Males	Females		
Feeding time (days)					
Larvae	4.53 (±1.24)	4.24 (±1.03)	4.26 (±0.98)	4	3–7[a]
Nymphs	4.84 (±1.40)	4.81 (±1.23)	4.56 (±1.17)	4	3–7[a]
Adult females	5.55 (±0.81)			5	4–8[a]
Eng. Weight (10^{-2} mg)					
Larvae	25.90	28.68	28.38	27	3.5–45
Nymphs	221.8	224.4	225.6	222.8	83–333
Adult females	4219			4250	1764–7288
Moulting time (days)					
Larvae	19.95	20.96	19.79	19	13–100
Nymphs	23.86	23.75	23.92	21	16–126
Hatched eggs					
All adult females	168 (±133)			180	0–445
Excluding non-laying ticks	211 (±115)			210	1–445

[a]Limited by experimental design.

Fig. S1. Distribution of moulting time in larvae and nymphs. Only ticks checked daily were included. Vertical lines show the median for larvae (L mdn) and for nymphs (N mdn).



3. Predictors of tick success

3.1. Attachment success

Model adult females

Family: Bernoulli; links: mu = logit
Formula: SuccessFirsAtt ~ scale(Fasting_time) + scale(Feed_time) + scale(Weight) + scale(Moult_time) + Y_EMER + (1|CLUTCH) + (1|Nest_ID) + (1|Nestling_ID)
Samples: 4 chains, each with iter = 85000; warmup = 42500; thin = 20; total post-warmup samples = 8500

Table S3. Model results for attachment success of adult females with lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, we report the potential scale reduction factor on split chains (Rhat, 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS)[1].

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Adult females (N = 303)							
Group-level effects:							
Clutch	1.14	0.36	0.48	1.91	1.00	8556	8265
Nest identity	0.42	0.29	0.02	1.07	1.00	8510	8617
Nestling identity	0.87	0.45	0.08	1.80	1.00	8393	8275
Population-level effects:							
Intercept	0.06	0.38	-0.70	0.80	1.00	8206	8158
Feeding time	0.10	0.19	-0.27	0.47	1.00	8266	8049
Eng. weight	-0.08	0.19	-0.45	0.29	1.00	8300	8395
Moulting time	**0.41**	0.21	**0.03**	**0.86**	1.00	8432	8229
Year:2019	0.41	0.58	-0.69	1.60	1.00	8539	8389
Fasting time	0.36	0.25	-0.13	0.86	1.00	8393	7596

3.2. Feeding success

Model nymphs

Family: bernoulli; links: mu = logit
Formula: Feeding_Success ~ scale(Feed_time) + scale(Weight) + scale(Moult_time) + Year + scale(Fasting_time) + (1|BATCH) + (1|CLUTCH) + (1|Feed_event)
Samples: 4 chains, each with iter = 110000; warmup = 55000; thin = 25; total post-warmup samples = 8800

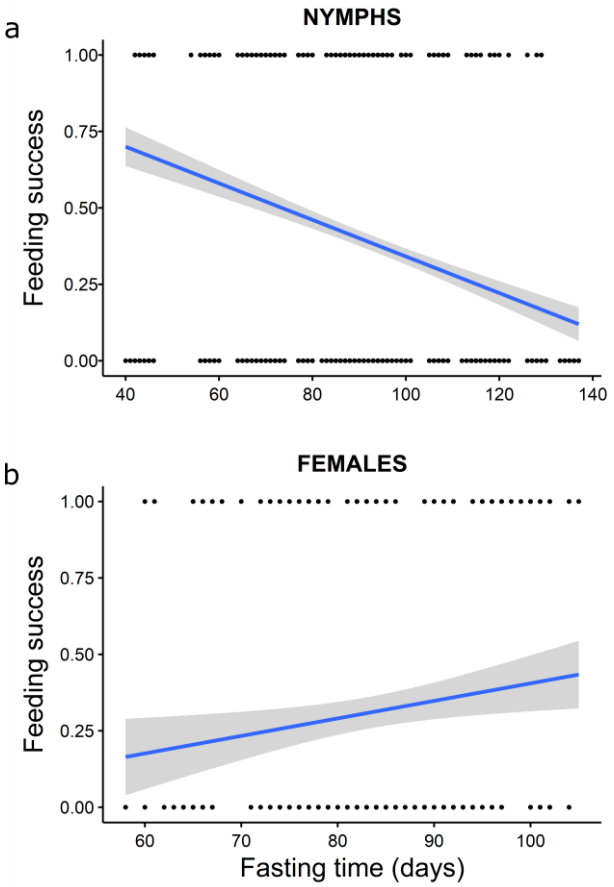
Model adult females

Family: Bernoulli; links: mu = logit
Formula: Feeding_Success ~ scale(Feed_time) + scale(Weight) + scale(Moult_time) + Year + scale(Fasting_time) + (1|NEST_ID) + (1|CLUTCH) + (1|Nestling_ID)
Samples: 4 chains, each with iter = 130000; warmup = 65000; thin = 30; total post-warmup samples = 8667

Table S4. Model results for feeding success of nymphs and adult females with lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, we report the potential scale reduction factor on split chains (Rhat, 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Nymphs (N = 1339)							
Group-level effects:							
Batch	1.92	0.73	0.96	3.73	1.00	8828	8620
Clutch	0.40	0.20	0.03	0.74	1.00	7887	8569
Feeding event	0.36	0.19	0.02	0.71	1.00	7640	8419
Population-level effects:							
Intercept	-0.05	1.03	-2.10	2.01	1.00	9006	8774
Feeding time	-0.04	0.07	-0.18	0.09	1.00	8627	8814
Eng. weight	0.05	0.07	-0.10	0.20	1.00	8744	8728
Moulting time	-0.00	0.08	-0.15	0.15	1.00	8929	8168
Year:2019	-1.12	1.58	-4.24	2.03	1.00	9129	8489
Fasting time	**-1.73**	0.15	**-2.03**	**-1.45**	1.00	8965	8335
Adult females (N = 303)							
Group-level effects:							
Clutch	0.68	0.38	0.05	1.49	1.00	8434	7892
Nest identity	0.68	0.36	0.05	1.41	1.00	8272	8605
Nestling identity	0.83	0.48	0.05	1.86	1.00	8148	8463
Population-level effects:							
Intercept	-0.95	0.41	-1.85	-0.24	1.00	8777	8111
Feeding time	0.11	0.19	-0.26	0.48	1.00	8104	8592
Eng. weight	0.13	0.18	-0.23	0.50	1.00	8510	8092
Moulting time	**0.37**	0.20	**0.00**	**0.79**	1.00	8824	8228
Year:2019	-0.33	0.57	-1.43	0.83	1.00	8628	8052
Fasting time	**0.59**	0.27	**0.08**	**1.12**	1.00	8959	8501

Fig. S2. Correlation between feeding success and fasting time. Data on nymphs (a) and adult females (b). In grey, 95% confidence intervals.



3.3. Moulting success

Model larvae

Family: bernoulli; links: mu = logit
Formula: Moult_Success ~ scale(Feed_time) + scale(Weight) + Year + scale(Fasting_time) + (1|BATCH) + (1|CLUTCH) + (1|Feed_event)
Samples: 4 chains, each with iter = 150000; warmup = 75000; thin = 35; total post-warmup samples = 8572

Model nymphs

Family: bernoulli; links: mu = logit
Formula: Moult_Success ~ scale(Feed_time) + scale(Weight) + Year + scale(Fasting_time) + (1|BATCH) + (1|CLUTCH) + (1|Feed_event)
Samples: 4 chains, each with iter = 65000; warmup = 32500; thin = 15; total post-warmup samples = 8667

Table S5. Model results for moulting success of larvae and nymphs with lower (l-95% CI), upper (u-95% CI) 95% credible intervals, and number of observations (N). On the right, we report the potential scale reduction factor on split chains (Rhat, 1 at convergence between chains), effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae (N = 3346)							
Group-level effects:							
Batch	2.23	0.81	1.19	4.25	1.00	8574	8438
Clutch	0.34	0.18	0.02	0.67	1.00	8338	8312
Feeding event	0.46	0.18	0.06	0.76	1.00	7942	8175
Population-level effects:							
Intercept	2.03	1.18	-0.46	4.35	1.00	8879	8559
Feeding time	**-0.46**	0.06	**-0.59**	**-0.34**	1.00	8847	8775
Eng. weight	**2.38**	0.10	**2.19**	**2.58**	1.00	8379	8207
Year:2019	-0.30	1.85	-4.05	3.31	1.00	8829	8483
Fasting time	**-0.40**	0.18	**-0.75**	**-0.04**	1.00	8063	8004
Nymphs (N = 531)							
Group-level effects:							
Batch	1.49	1.32	0.07	4.72	1.00	8135	8378
Clutch	1.07	0.82	0.05	3.06	1.00	8112	8000
Feeding event	1.34	0.93	0.06	3.56	1.00	8353	8420
Population-level effects:							
Intercept	7.87	2.30	4.54	13.49	1.00	8553	8151
Feeding time	**-3.31**	0.97	**-5.60**	**-1.94**	1.00	8081	7873
Eng. weight	**1.52**	0.53	**0.63**	**2.70**	1.00	8491	8540
Year:2019	2.43	2.45	-3.05	6.68	1.00	7663	8107
Fasting time	-0.38	0.50	-1.46	0.51	1.00	8532	8380

3.4. Survival success

Model nymphs

Family: bernoulli; links: mu = logit
Formula: Survival_Success ~ scale(Feed_time) + scale(Weight) + Scale(Moult_time) + Year + scale(Fasting_time) + (1|BATCH) + (1|CLUTCH) + (1|Feed_event)
Samples: 4 chains, each with iter = 130000; warmup = 65000; thin = 30; total post-warmup samples = 8667

Model adult females

Family: bernoulli; links: mu = logit
Formula: Survival_Success ~ scale(Feed_time) + scale(Weight) + scale(Moult_time) + Year + scale(Fasting_time) + (1|NEST_ID) + (1|CLUTCH) + (1|Nestling_ID)
Samples: 4 chains, each with iter = 650000; warmup = 325000; thin = 150; total post-warmup samples = 8667

Table S6. Model results for survival success of nymphs and adult females with lower (l-95% CI), upper (u-95% CI) 95% credible intervals, and number of observations (N). On the right, we report the potential scale reduction factor on split chains (Rhat, 1 at convergence between chains), effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Nymphs (N = 1338)							
Group-level effects:							
Batch	1.77	0.67	0.89	3.47	1.00	8951	8927
Clutch	0.35	0.18	0.02	0.68	1.00	7788	8317
Feeding event	0.34	0.18	0.02	0.67	1.00	8361	8421
Population-level effects:							
Intercept	-0.16	0.94	-1.98	1.81	1.00	8717	8687
Feeding time	-0.07	0.07	-0.20	0.07	1.00	8506	8391
Eng. weight	0.07	0.07	-0.08	0.21	1.00	8837	8339
Moulting time	-0.01	0.08	-0.16	0.13	1.00	8656	8794
Year:2019	-1.08	1.44	-4.05	1.82	1.00	8715	8466
Fasting time	**-1.64**	0.15	**-1.93**	**-1.36**	1.00	8556	8510
Adult females (N = 303)							
Group-level effects:							
Clutch	0.90	0.49	0.07	1.98	1.00	8291	8586
Nest identity	0.91	0.48	0.07	1.91	1.00	8307	8680
Nestling identity	1.24	0.63	0.13	2.62	1.00	7777	7952
Population-level effects:							
Intercept	-1.57	0.54	-2.82	-0.66	1.00	8289	8448
Feeding time	-0.02	0.23	-0.47	0.42	1.00	8758	8047
Eng. weight	0.13	0.23	-0.30	0.59	1.00	8379	7661
Moulting time	0.41	0.24	-0.04	0.91	1.00	8652	8544
Year:2019	-0.22	0.70	-1.59	1.15	1.00	8269	8359
Fasting time	**0.68**	0.33	**0.08**	**1.37**	1.00	8893	8413

3.5. Egg-laying success

Model egg-laying success

Family: bernoulli; links: mu = logit
Formula: Laying_Success ~ scale(Feed_time) + scale(Weight) + scale(Infest_attempt) + Year + (1|CLUTCH) + (1|Nestling_ID) + (1|NEST_ID)
Samples: 4 chains, each with iter = 110000; warmup = 55000; thin = 25; total post-warmup samples = 8800

Table S7. Model results for egg-laying success in adult females with lower (l-95% CI), upper (u-95% CI) 95% credible intervals, and number of observations (N). Infestation attempt refers to ticks attaching at the first or second infestation. On the right, we report the potential scale reduction factor on split chains (Rhat, 1 at convergence between chains), effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Adult females (N = 163)							
Group-level effects:							
Clutch	2.16	1.88	0.09	6.90	1.00	8051	8553
Nest identity	2.63	2.09	0.14	7.82	1.00	9013	8435
Nestling identity	2.58	2.18	0.12	7.98	1.00	8822	8689
Population-level effects:							
Intercept	9.36	5.27	3.35	23.27	1.00	7861	8217
Feeding time	-0.48	0.71	-2.03	0.80	1.00	8235	8439
Eng. weight	-0.85	0.88	-2.95	0.48	1.00	8882	8382
Infest. attempt	**-1.54**	1.02	**-4.15**	**-0.21**	1.00	8738	8717
Year:2018	-2.45	3.47	-10.58	3.21	1.00	8643	8280
Year:2019	-3.45	3.56	-12.35	1.79	1.00	8462	8299

4. Phenotypic correlations across stages

Table S8. Phenotypic correlations of the same trait across life stages. Tau Kendall's correlations on standardised and normalized data. In round brackets, 95% confidence intervals on 10^4 bootstrap iterations. Similar results were obtained when raw data were used. $^{*}P < 0.05$, $^{**}P < 0.001$.

	Nymphs	Adult females
Feeding time		
Larvae	-0.028 (-0.106, 0.050)	0.133 (-0.031, 0.288)
Nymphs		-0.044 (-0.199, 0.114)
Engorgement weight		
Larvae	0.182 (0.126, 0.239)**	0.130 (0.007, 0.251)*
Nymphs		0.460 (0.368, 0.542)**
Moulting time		
Larvae	0.048 (-0.016, 0.112)	

Fig. S3. Correlation between feeding time and moulting time. Data on larvae (a) and nymphs (b). In grey, 95% confidence intervals.

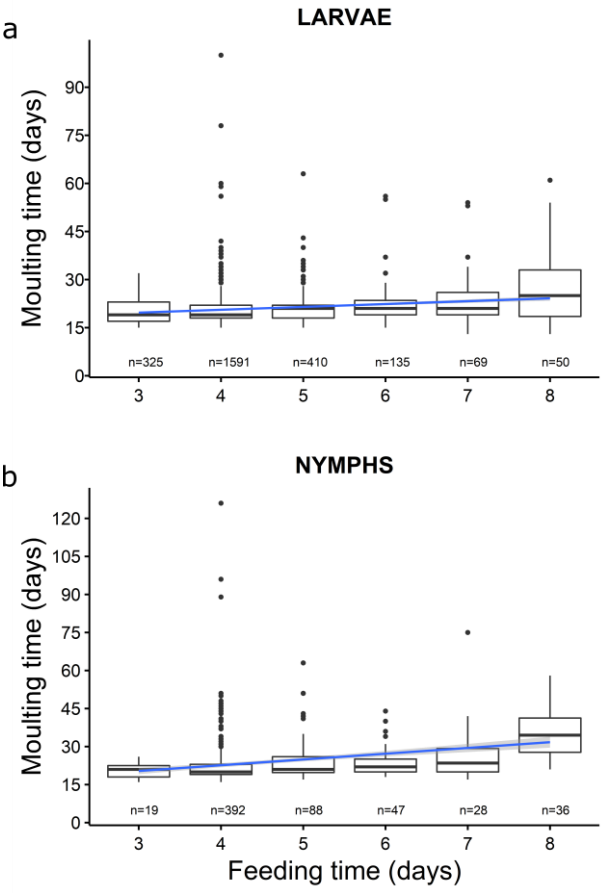
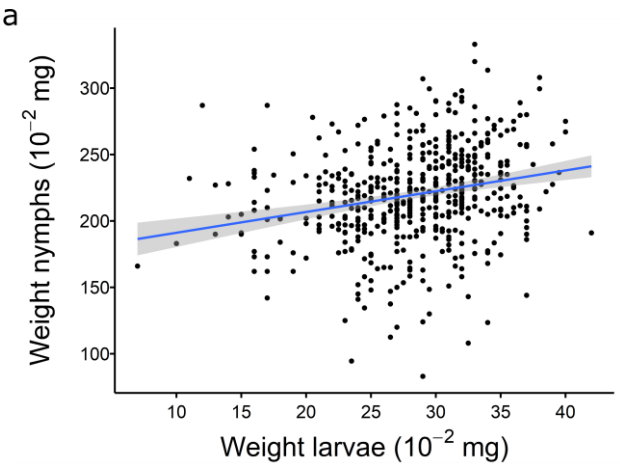
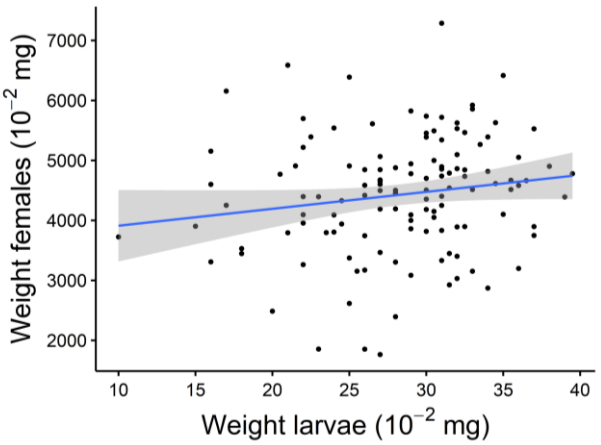


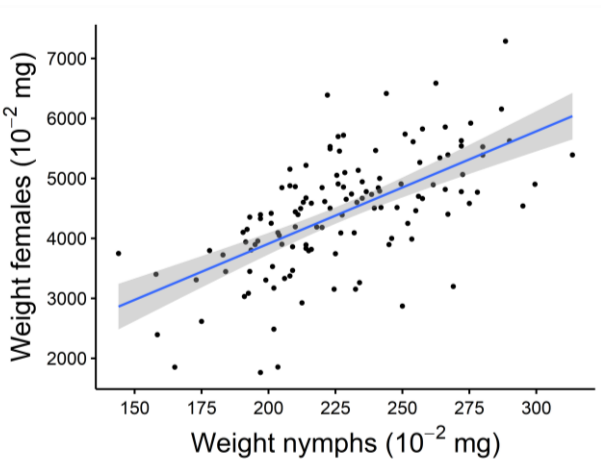
Fig. S4. Phenotypic correlation of engorgement weight across life stages. Larvae and nymphs (a), larvae and adult females (b), nymphs and adult females (c). In grey, 95% confidence intervals.



b



c



5. Animal Models

5.1. Larvae

```
Moult_TimeL <- scale((LarvaeDataALL$MoultTime)^(-1.9))
Feed_TimeL <- scale((LarvaeDataALL$FeedTime)^(-1))
WeightL <- scale(LarvaeDataALL$ENG_WEIGHTL)

priorExt <- list(R=list(V=diag(3),nu=0.003), G=list(G1=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G2=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G3=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G4=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000)))

library(parallel)
setCores <- 5
cl <- makeCluster(getOption("cl.cores",setCores))
cl.pkg <- clusterEvalQ(cl,library(MCMCglmm))
clusterExport(cl,"priorExt")
```

```
clusterExport(cl,"LarvaeDataALL")
clusterExport(cl,"pedInclus")
clusterExport(cl,"Moult_TimeL ")
clusterExport(cl,"Feed_TimeL ")
clusterExport(cl,"WeightL")

LARVAE <- parLapply(cl=cl,1:5, function(i) [MCMCglmm(fixed=cbind(WeightL, Moult_TimeL,
Feed_TimeL) ~ trait + trait:SEX + trait:Fasting_timeL-1, random=~us(trait):animal +
idh(trait):CLUTCH + idh(trait):FEID + idh(trait):BATCH, rcov= ~us(trait):units,
family=c("gaussian","gaussian","gaussian"), thin=1100, nitt=8800000, burnin=2500,
data=LarvaeDataALL, prior=priorExt, pedigree=pedInclus, saveX=T, saveZ=T)])
stopCluster(cl)
```

Model summary (3rd chain)

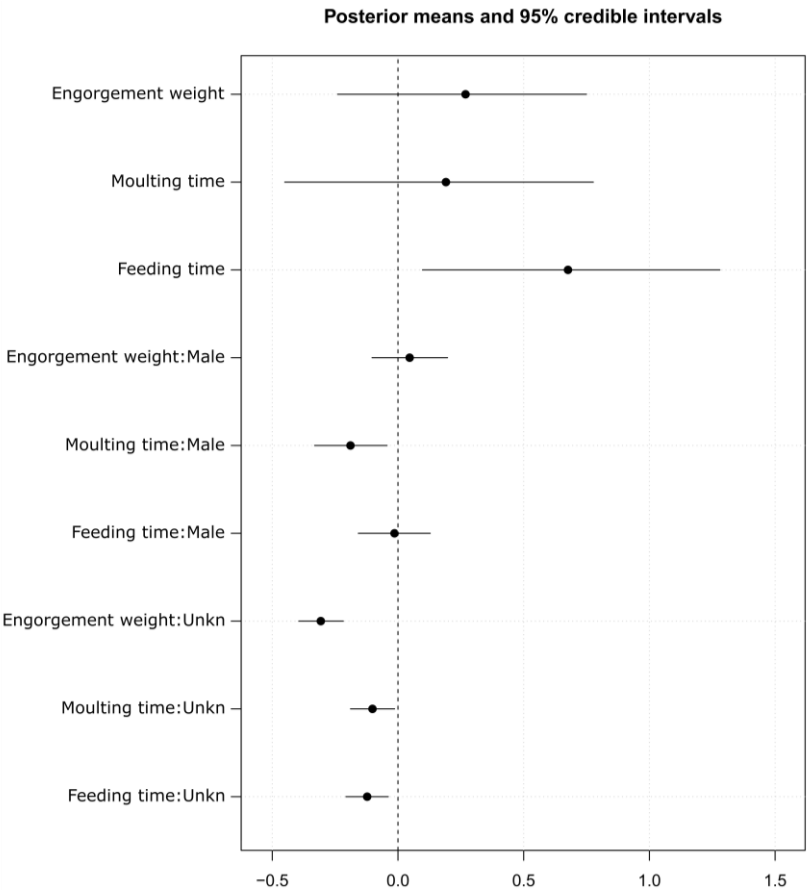
Iterations = 2501:8799201
Thinning interval = 1100
Sample size = 7998
DIC: 23220.63

Table S9. Model output for larvae with 95% credible intervals (95% CI). Effective sample size adjusted for autocorrelation (eff. samp.) and posterior probability associated to the event (pMCMC) are shown on the right.

Variable	Post. mean	Lower 95% CI	Upper 95% CI	Eff. samp.	pMCMC
Pedigree	**G-structure: ~us(trait):animal**				
Weight:Weight.animal	0.100	5.078e-06	0.188	7998	
Moult_time:Weight.animal	0.076	-1.719e-03	0.154	6469	
Feed_time:Weight.animal	0.032	-1.574e-02	0.099	5054	
Weight:Moult_time.animal	0.076	-1.719e-03	0.154	6469	
Moult_time:Moult_time.animal	0.152	1.360e-06	0.302	7998	
Feed_time:Moult_time.animal	0.032	-2.884e-02	0.110	6068	
Weight:Feed_time.animal	0.032	-1.574e-02	0.099	5054	
Moult_time:Feed_time	0.032	-2.884e-02	0.110	6068	
Feed_time:Feed_time	0.050	4.231e-10	0.145	7998	
Maternal effect	**~idh(trait):CLUTCH**				
Weight.CLUTCH	0.014	1.773e-10	0.044	7998	
Moult_time.CLUTCH	0.044	1.429e-09	0.107	7998	
Feed_time.CLUTCH	0.017	6.707e-10	0.059	7998	
Feeding event	**~idh(trait):FEID**				
Weight.FEID	0.041	0.012	0.072	7725	
Moult_time.FEID	0.059	0.010	0.112	8943	
Feed_time.FEID	0.182	0.114	0.252	8804	

Batch	**~idh(trait):BATCH**				
Weight.BATCH	0.259	0.026	0.712	7998	
Moult_time.BATCH	0.395	0.038	1.093	7998	
Feed_time.BATCH	0.318	0.034	0.857	7998	
Residual	**R-structure: ~us(trait):units**				
Weight:Weight.units	0.689	0.629	0.748	8297	
Moult_time:Weight.units	0.146	0.090	0.200	7525	
Feed_time:Weight.units	0.039	-0.000	0.076	5720	
Weight:Moult_time.units	0.146	0.090	0.200	7525	
Moult_time:Moult_time.units	0.573	0.482	0.656	7998	
Feed_time:Moult_time.units	0.065	0.021	0.109	6782	
Weight:Feed_time.units	0.039	-0.000	0.076	5720	
Moult_time:Feed_time.units	0.065	0.021	0.109	6782	
Feed_time:Feed_time.units	0.639	0.578	0.693	7998	
Response variables					
Weight	0.266	-0.240	0.738	7998	0.273
Moult_time	0.190	-0.481	0.743	8500	0.498
Feed_time	0.679	0.091	1.269	7998	0.025
Fixed effects					
Weight:Male	0.045	-0.105	0.189	8269	0.550
Moult_time:Male	-0.190	-0.335	-0.048	7998	0.010
Feed_time:Male	-0.013	-0.161	0.122	7998	0.849
Weight:Unknown	-0.307	-0.395	-0.219	7912	<1e-04
Moult_time:Unknown	-0.102	-0.186	-0.014	7998	0.022
Feed_time:Unknown	-0.123	-0.207	-0.036	7998	0.006
Weight:Fasting	-0.000	-0.003	0.003	7998	0.912
Moult_time:Fasting	-0.002	-0.006	0.002	7998	0.273
Feed_time:Fasting	-0.006	-0.010	-0.002	8265	0.002

Fig. S5. Posterior means and 95% credible intervals from the Animal Model on larvae (see also Table S9). The three response variables (engorgement weight, moulting time, feeding time) are showed on top and followed by the fixed effect of sex. The effect of fasting time was excluded from the plot for graphical clarity.



Model without "SEX" as fixed effect (larvae)

```
Moult_TimeL <- scale((LarvaeDataALL$MoultTime)^(-1.9))
Feed_TimeL <- scale((LarvaeDataALL$FeedTime)^(-1))
WeightL <- scale(LarvaeDataALL$ENG_WEIGHTL)

priorExt <- list(R=list(V=diag(3),nu=0.003), G=list(G1=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G2=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G3=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G4=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000)))

library(parallel)
setCores <- 5
```

```
cl <- makeCluster(getOption("cl.cores",setCores))
cl.pkg <- clusterEvalQ(cl,library(MCMCglmm))
clusterExport(cl,"priorExt")
clusterExport(cl,"LarvaeDataALL")
clusterExport(cl,"pedInclus")
clusterExport(cl,"Moult_TimeL ")
clusterExport(cl,"Feed_TimeL ")
clusterExport(cl,"WeightL")

LARVAEnoSex <- parLapply(cl=cl,1:5, function(i) [MCMCglmm(fixed=cbind(WeightL,
Moult_TimeL, Feed_TimeL) ~ trait + trait:Fasting_timeL-1, random=~us(trait):animal +
idh(trait):CLUTCH + idh(trait):FEID + idh(trait):BATCH, rcov= ~us(trait):units,
family=c("gaussian", "gaussian", "gaussian"), thin=1100, nitt=8800000, burnin=2500,
data=LarvaeDataALL, prior=priorExt, pedigree=pedInclus, saveX=T, saveZ=T)])
stopCluster(cl)
```

Model summary (3rd chain)

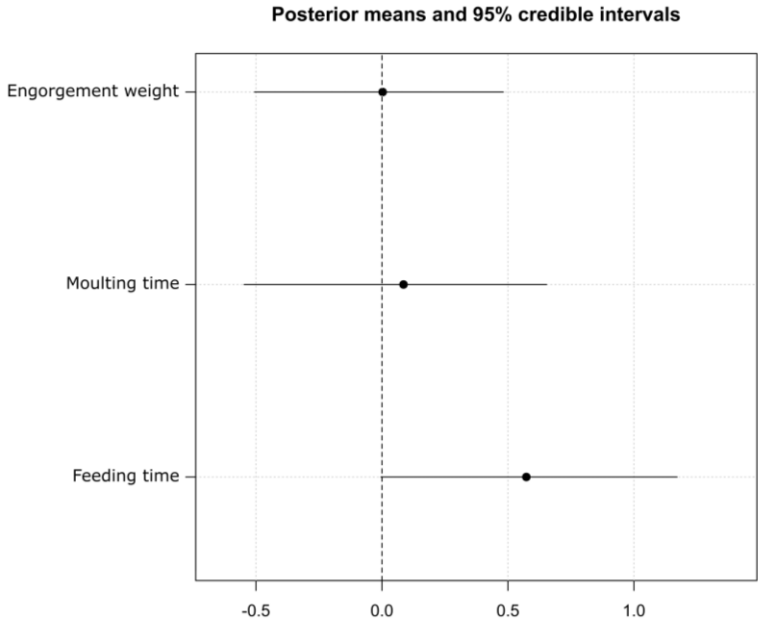
Iterations = 2501:8799201
Thinning interval = 1100
Sample size = 7998
DIC: 23279.16

Table S10. Model output for larvae without "SEX" in the fixed effects with 95% credible intervals (95% CI). Effective sample size adjusted for autocorrelation (eff. samp.) and posterior probability associated to the event (pMCMC) are shown on the right.

Variable	Post. mean	Lower 95% CI	Upper 95% CI	Eff. samp.	pMCMC
Pedigree	**G-structure: ~us(trait):animal**				
Weight:Weight.animal	0.115	2.386e-02	0.219	7998	
Moult_time:Weight.animal	0.085	-2.130e-04	0.166	6478	
Feed_time:Weight.animal	0.037	-1.674e-02	0.109	6263	
Weight:Moult_time.animal	0.085	-2.130e-04	0.166	6478	
Moult_time:Moult_time.animal	0.158	2.579e-06	0.309	7613	
Feed_time:Moult_time.animal	0.036	-2.680e-02	0.116	6043	
Weight:Feed_time.animal	0.037	-1.674e-02	0.109	6263	
Moult_time:Feed_time	0.036	-2.680e-02	0.116	6043	
Feed_time:Feed_time	0.051	2.392e-10	0.148	7998	
Maternal effect	**~idh(trait):CLUTCH**				
Weight.CLUTCH	0.013	1.662e-09	0.043	7998	
Moult_time.CLUTCH	0.046	2.095e-09	0.108	7998	
Feed_time.CLUTCH	0.016	1.799e-12	0.058	8293	
Feeding event	**~idh(trait):FEID**				
Weight.FEID	0.040	0.011	0.071	7998	
Moult_time.FEID	0.056	0.010	0.111	7998	
Feed_time.FEID	0.183	0.117	0.250	7998	

Batch	**~idh(trait):BATCH**				
Weight.BATCH	0.271	0.025	0.742	7998	
Moult_time.BATCH	0.383	0.034	1.039	6693	
Feed_time.BATCH	0.317	0.039	0.846	7360	
Residual	**R-structure: ~us(trait):units**				
Weight:Weight.units	0.695	0.633	0.755	7998	
Moult_time:Weight.units	0.145	0.090	0.202	7998	
Feed_time:Weight.units	0.041	-0.003	0.078	6845	
Weight:Moult_time.units	0.145	0.090	0.202	7998	
Moult_time:Moult_time.units	0.573	0.482	0.659	7998	
Feed_time:Moult_time.units	0.065	0.019	0.111	6881	
Weight:Feed_time.units	0.041	-0.003	0.078	6845	
Moult_time:Feed_time.units	0.065	0.019	0.111	6881	
Feed_time:Feed_time.units	0.640	0.583	0.695	8197	
Response variables					
Weight	0.000	-0.488	0.494	7998	0.986
Moult_time	0.085	-0.501	0.675	7998	0.735
Feed_time	0.572	-0.057	1.125	7998	0.058
Fixed effects					
Weight:Fasting	-0.000	-0.003	0.003	7998	0.992
Moult_time:Fasting	-0.002	-0.006	0.002	7998	0.279
Feed_time:Fasting	-0.006	-0.010	-0.003	7998	**0.002**

Fig. S6. Posterior means and 95% credible intervals from the Animal Model on larvae without "SEX" as fixed effect (see also Table S10). The three response variables (engorgement weight, moulting time, feeding time) are showed. The effect of fasting time was excluded from the plot for graphical clarity.



5.2. Nymphs

```
Moult_TimeN <- scale((NymphsData$MoultTime)^(-3))
Feed_TimeN <- scale((NymphsData$FeedTime)^(-1.5))
WeightN <- scale(NymphsData$ENG_WEIGHT)

priorExt <- list(R=list(V=diag(3),nu=0.003), G=list(G1=list(V=diag(3),nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G2=list(V=diag(3),nu=3, alpha.mu=
c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G3=list(V=diag(3),nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G4=list(V=diag(3),nu=3,
alpha.mu=c(0.001,0.001,0.001),alpha.V=diag(3)*1000)))

library(parallel)
setCores <- 5
cl <- makeCluster(getOption("cl.cores",setCores))
cl.pkg <- clusterEvalQ(cl,library(MCMCglmm))
clusterExport(cl,"priorExt")
clusterExport(cl,"NymphsData")
clusterExport(cl,"pedInclus")
clusterExport(cl,"Moult_TimeN")
clusterExport(cl,"Feed_TimeN")
clusterExport(cl,"WeightN")
NYMPHS <- parLapply(cl=cl,1:5, function(i) [MCMCglmm(fixed=cbind(WeightN, Moult_TimeN,
Feed_TimeN) ~ trait + trait:SEX + trait:Fasting_timevN-1, random= ~us(trait):animal +
idh(trait):CLINF + idh(trait):FEID + idh(trait):BATCH, rcov= ~us(trait):units,
family=c("gaussian","gaussian","gaussian"), thin=1000, nitt=9100000, burnin=2500,
data=NymphsData, prior=priorExt, pedigree=pedInclus, saveX=T, saveZ=T)])
stopCluster(cl)
```

Model summary (3rd chain)

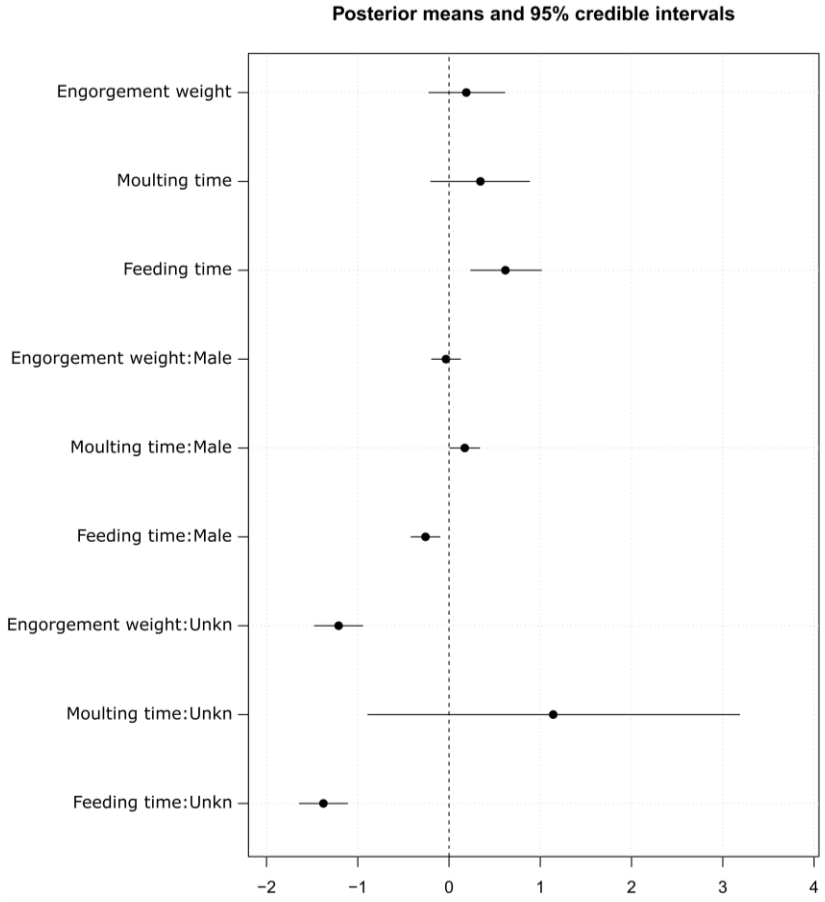
Iterations = 2501:9099501
Thinning interval = 1000
Sample size = 9098
DIC: 4604.469

Table S11. Model output for nymphs with 95% credible intervals (95% CI). Effective sample size adjusted for autocorrelation (eff. samp.) and posterior probability associated to the event (pMCMC) are shown on the right.

Variable	Post. mean	Lower 95% CI	Upper 95% CI	Eff. samp.	pMCMC
Pedigree	**G-structure: ~us(trait):animal**				
Weight:Weight.animal	0.383	9.909e-02	0.674	9098	
Moult_time:Weight.animal	0.136	-1.939e-02	0.297	8085	
Feed_time:Weight.animal	0.011	-9.167e-02	0.119	7594	
Weight:Moult_time.animal	0.136	-1.939e-02	0.297	8085	

Moult_time:Moult_time.animal	0.334	1.197e-01	0.538	9098	
Feed_time:Moult_time.animal	0.050	-3.673e-02	0.165	7566	
Weight:Feed_time.animal	0.011	-9.167e-02	0.119	7594	
Moult_time:Feed_time	0.050	-3.673e-02	0.165	7566	
Feed_time:Feed_time	0.062	2.281e-09	0.185	8572	
Maternal effect	**~idh(trait):CLUTCH**				
Weight.CLUTCH	0.060	4.660e-10	0.166	9098	
Moult_time.CLUTCH	0.038	2.280e-09	0.105	9650	
Feed_time.CLUTCH	0.043	9.703e-11	0.108	9098	
Feeding event	**~idh(trait):FEID**				
Weight.FEID	0.081	0.027	0.140	9098	
Moult_time.FEID	0.060	0.005	0.117	9098	
Feed_time.FEID	0.088	0.031	0.150	9098	
Batch	**~idh(trait):BATCH**				
Weight.BATCH	0.021	1.535e-12	0.081	9098	
Moult_time.BATCH	0.232	2.057e-02	0.601	7895	
Feed_time.BATCH	0.015	3.683e-10	0.059	8613	
Residual	**R-structure: ~us(trait):units**				
Weight:Weight.units	0.440	0.270	0.620	9098	
Moult_time:Weight.units	0.174	0.062	0.281	8163	
Feed_time:Weight.units	0.136	0.051	0.228	7703	
Weight:Moult_time.units	0.174	0.062	0.281	8163	
Moult_time:Moult_time.units	0.526	0.383	0.670	9098	
Feed_time:Moult_time.units	0.228	0.126	0.321	8283	
Weight:Feed_time.units	0.136	0.051	0.228	7703	
Moult_time:Feed_time.units	0.228	0.126	0.321	8283	
Feed_time:Feed_time.units	0.656	0.531	0.772	7638	
Response variables					
Weight	1.624e-01	-3.441e-01	6.180e-01	8168	0.496
Moult_time	1.640e-01	-4.521e-01	7.448e-01	9098	0.572
Feed_time	6.286e-01	1.946e-01	1.070e+00	9098	**0.006**
Fixed effects					
Weight:Male	-3.202e-02	-1.907e-01	1.250e-01	9098	0.697
Moult_time:Male	1.726e-01	1.572e-02	3.367e-01	9098	**0.035**
Feed_time:Male	-2.530e-01	-4.127e-01	-1.014e-01	9098	**0.001**
Weight:Unknown	-1.210e+00	-1.499e+00	-9.548e-01	9098	**<1e-04**
Moult_time:Unknown	1.068e+00	-9.314e-01	3.122e+00	8654	0.293
Feed_time:Unknown	-1.444e+00	-1.709e+00	-1.182e+00	9855	**<1e-04**
Weight:Fasting	6.202e-05	-5.116e-03	4.882e-03	8496	0.993
Moult_time:Fasting	-2.885e-03	-8.136e-03	2.224e-03	9376	0.279
Feed_time:Fasting	-4.871e-03	-9.483e-03	-6.459e-05	9098	**0.040**

Fig. S7. Posterior means and 95% credible intervals from the Animal Model on nymphs (see also Table S11). The three response variables (engorgement weight, moulting time, feeding time) are showed on top and followed by the fixed effect of sex. The effect of fasting time was excluded from the plot for graphical clarity.



5.3. Adult females

```
Hatch_eggsF <- scale(FemalesData$HATCH_EGGS)
Feed_TimeF <- scale((FemalesData$FeedTime)^(-0.7))
WeightF <- scale(FemalesData$ENG_WEIGHT)

prior <- list(R=list(V=diag(3), nu=0.003), G=list(G1=list(V=diag(3), nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G2=list(V=diag(3),nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G3=list(V=diag(3), nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000), G4=list(V=diag(3), nu=3,
alpha.mu=c(0.001,0.001,0.001), alpha.V=diag(3)*1000)))

library(parallel)
setCores <- 5
```

```
cl <- makeCluster(getOption("cl.cores",setCores))
cl.pkg <- clusterEvalQ(cl,library(MCMCglmm))
clusterExport(cl,"priorExt")
clusterExport(cl,"FemalesData")
clusterExport(cl,"pedInclus")
clusterExport(cl,"Hatch_eggsF")
clusterExport(cl,"Feed_TimeF")
clusterExport(cl,"WeightF")

FEMALES <- parLapply(cl=cl,1:5, function(i) [MCMCglmm(fixed=cbind(WeightF, Hatch_eggsF,
Feed_TimeF) ~ trait + trait:Fasting_timeF + trait:YEAR-1, random= ~us(trait):animal +
idh(trait):CLUTCH + idh(trait):Nest_ID + idh(trait):Host_ID, rcov= ~us(trait):units,
family=c("gaussian","gaussian","gaussian"), thin=5200, nitt=44500000, burnin=16000,
data=FemalesData, prior=prior, pedigree=pedInclus, saveX=T, saveZ=T)])
stopCluster(cl)
```

Model summary (3rd chain)

Iterations = 16001:44496801
Thinning interval = 5200
Sample size = 8555
DIC: -274.439

Table S12. Model output for adult females with 95% credible intervals (95% CI). Effective sample size adjusted for autocorrelation (eff. samp.) and posterior probability associated to the event (pMCMC) are shown on the right.

Variable	Post. mean	Lower 95% CI	Upper 95% CI	Eff. samp.	pMCMC
Pedigree	**G-structure: ~us(trait):animal**				
Weight:Weight.animal	0.657	1.164e-01	1.166	7820	
Hatch_eggs:Weight.animal	0.193	-1.034e-01	0.557	6948	
Feed_time:Weight.animal	-0.118	-4.568e-01	0.186	6025	
Weight:Hatch_eggs.animal	0.193	-1.034e-01	0.557	6948	
Hatch_eggs:Hatch_eggs.animal	0.321	3.946e-07	0.802	8160	
Feed_time:Hatch_eggs.animal	0.042	-2.226e-01	0.327	7775	
Weight:Feed_time.animal	-0.118	-4.568e-01	0.186	6025	
Hatch_eggs:Feed_time	0.042	-2.226e-01	0.327	7775	
Feed_time:Feed_time	0.367	5.839e-10	0.812	7847	
Maternal effect	**~idh(trait):CLUTCH**				
Weight.CLUTCH	0.046	6.794e-12	0.154	8555	
Hatch_eggs.CLUTCH	0.054	1.534e-10	0.199	8555	
Feed_time.CLUTCH	0.294	1.214e-08	0.600	8555	
Nest Identity	**~idh(trait):Nest_ID**				
Weight.Nest_ID	0.043	6.849e-10	0.142	8555	
Hatch_eggs.Nest_ID	0.085	3.798e-10	0.261	8555	
Feed_time.Nest_ID	0.093	1.172e-07	0.271	8555	

Nestling Identity	**~idh(trait):Host_ID**				
Weight.Host_ID	0.052	9.829e-10	0.180	8856	
Hatch_eggs.Host_ID	0.067	1.351e-09	0.233	8555	
Feed_time.Host_ID	0.163	1.043e-08	0.444	8555	
Residual	**R-structure: ~us(trait):units**				
Weight:Weight.units	0.277	0.001	0.633	7805	
Hatch_eggs:Weight.units	0.023	-0.232	0.272	7229	
Feed_time:Weight.units	0.093	-0.140	0.343	6193	
Weight:Hatch_eggs.units	0.023	-0.232	0.272	7229	
Hatch_eggs:Hatch_eggs.units	0.473	0.039	0.832	8130	
Feed_time:Hatch_eggs.units	0.015	-0.214	0.239	7642	
Weight:Feed_time.units	0.093	-0.140	0.343	6193	
Hatch_eggs:Feed_time.units	0.015	-0.214	0.239	7642	
Feed_time:Feed_time.units	0.286	0.001	0.704	7313	
Response variables					
Weight	-0.754	-2.181	0.715	8873	0.305
Hatch_eggs	-1.904	-3.892	0.060	8011	0.056
Feed_time	-0.436	-2.081	1.168	8555	0.604
Fixed effects					
Weight:Fasting	0.003	-0.015	0.021	8875	0.765
Hatch_eggs:Fasting	0.007	-0.012	0.025	8555	0.483
Feed_time:Fasting	0.005	-0.016	0.025	9223	0.609
Weight:YEAR2018	0.799	0.450	1.173	8555	**<1e-04**
Hatch_eggs: YEAR2018	1.051	-0.380	2.432	7840	0.146
Feed_time: YEAR2018	-0.122	-0.603	0.353	8555	0.604
Weight:YEAR2019	0.732	0.264	1.190	8555	**0.002**
Hatch_eggs: YEAR2019	1.594	0.205	3.069	7884	**0.031**
Feed_time: YEAR2019	0.026	-0.546	0.589	8924	0.935

Fig. S8. Posterior means and 95% credible intervals from the Animal Model on adult females (see also Table S12). The three response variables (engorgement weight, number of hatched eggs, feeding time) are showed on top and followed by the fixed effect of year. The effect of fasting time was excluded from the plot for graphical clarity.

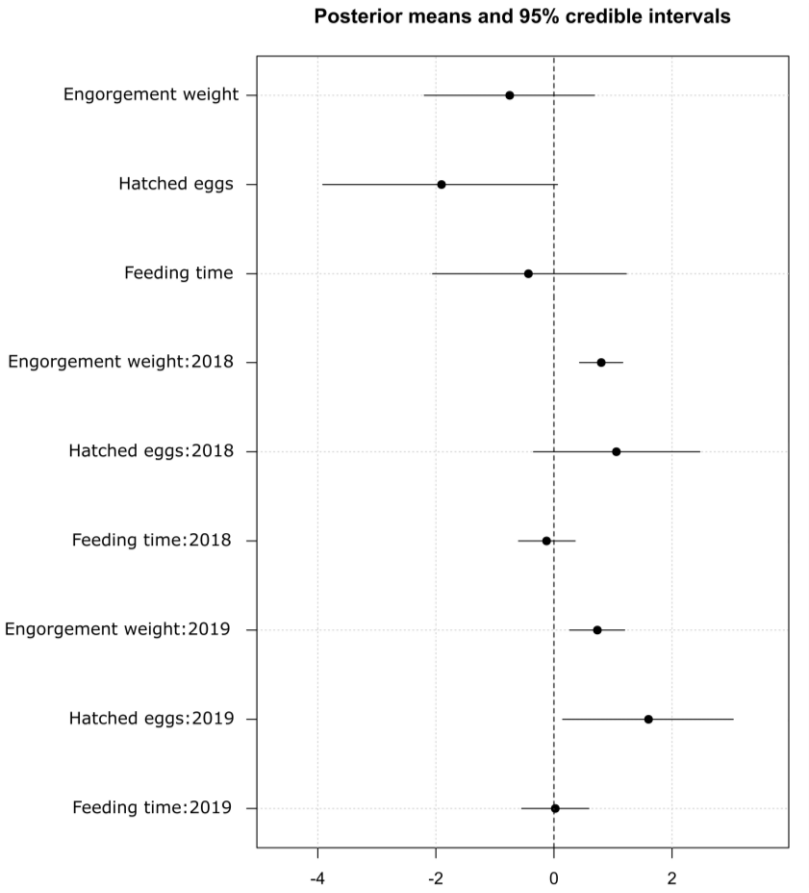
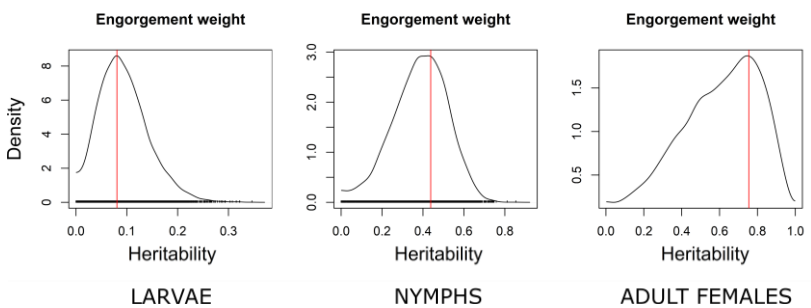


Fig. S9. Density distributions of heritability estimates for engorgement weight, feeding time, moulting time, and number of hatched eggs in larvae, nymphs, and adult females. In red, posterior mode of the distribution. A bell-shaped posterior distribution with a mode close to the 50%-quartile of the distribution was considered evidence of significant heritability.



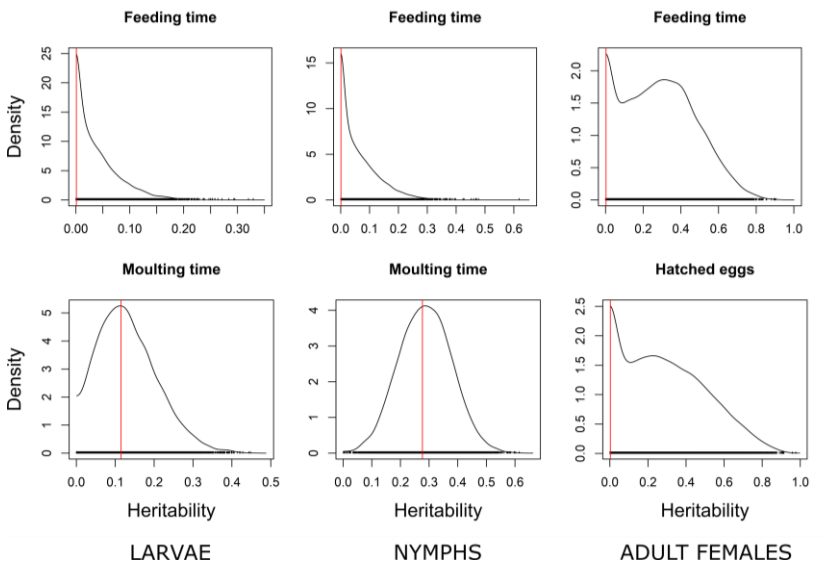
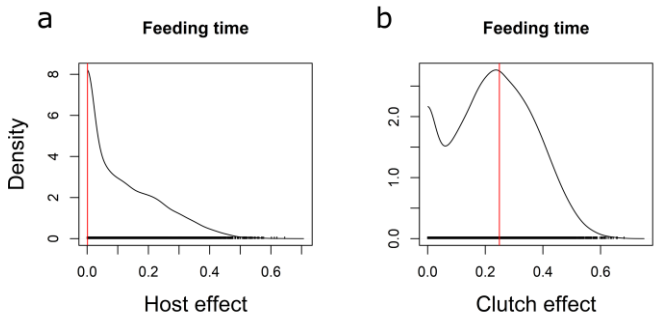


Fig. S10. Density distributions of host (nestling identity; a) and clutch effect (b) for feeding time in adult females. In red, posterior mode of the distribution. A bell-shaped posterior distribution with a mode close to the 50%-quartile of the distribution was considered evidence of significant heritability.



6. Estimates of heritability and evolvability

Estimates of heritability alone have a number of shortcomings when it comes to quantify and interpret the evolutionary potential of a trait (Hansen et al. 2011; Houle 1992; Wilson 2008), namely the population potential to generate heritable phenotypic variation that is potentially adaptive. For instance, heritability is scaled on the phenotypic variance and dependent on model specifications hampering comparison across studies. Hence, to provide a more thorough description of evolutionary potential for our traits, the coefficient of additive genetic variation (CV_a), the coefficient of residual variation (CV_r), and the mean-standardised additive variance (I_a) are also reported. We calculated them as follows.

$$CV_a = 100 \frac{\sqrt{V_a}}{\bar{X}}$$

$CV_r = 100\ \frac{\sqrt{V_r}}{\bar{X}}$ with $V_r = V_p - V_a$

$I_a = \left(\frac{CV_a}{100}\right)^2$

where V_a: additive genetic variance, V_p: total phenotypic variance, and $\bar{X}$: mean of the trait.

Table S13. Heritability on the observed scale and estimates of evolvability for nymphs. Heritability on the observed scale ($\boldsymbol{h_o^2}$), coefficients of additive genetic variation (**CV_a**), residual variation (**CV_r**), and mean-standardised additive variance (**I_a**).

	Feeding time	Engorgement weight	Moulting time
h_o^2	0.042	0.342	0.363
CV_a	5.965	10.214	25.305
CV_r	28.572	14.182	33.487
I_a	0.004	0.010	0.064

Supplementary information chapter IV

Fig. S1. Number of great tits infested by a different number of *I. arboricola* larvae in our study area. Only infested birds are plotted: 15 females (black) and 27 males (yellow).

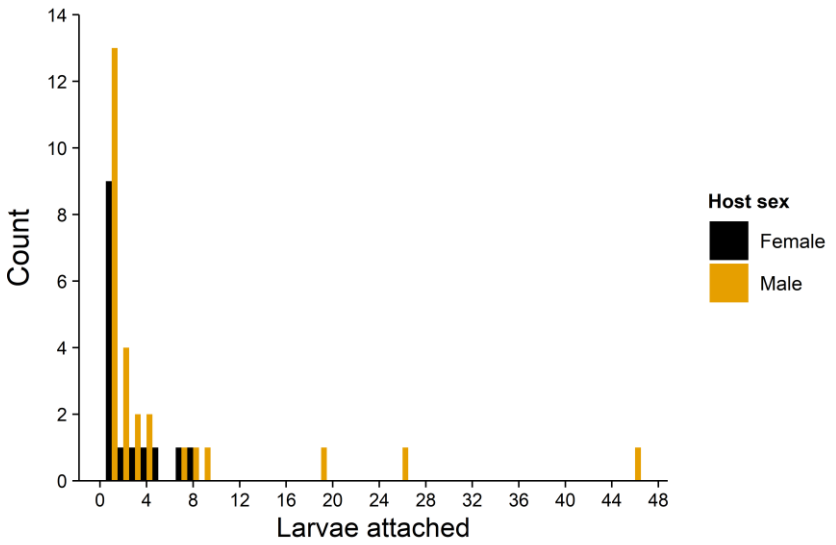


Fig. S2. Number of great tits infested by a different number of *I. arboricola* nymphs in our study area. Only infested birds are plotted: 21 females (black) and 16 males (yellow).

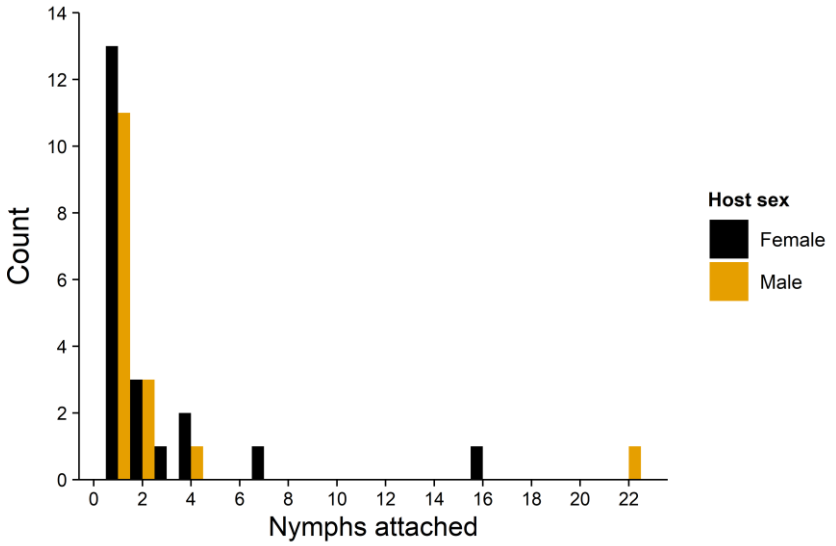


Fig. S3. Number of great tits infested by a different number of *I. arboricola* larvae and/or nymphs in our study area. Only infested birds are plotted: 78 individuals including those considered in fig. S1 and S2.

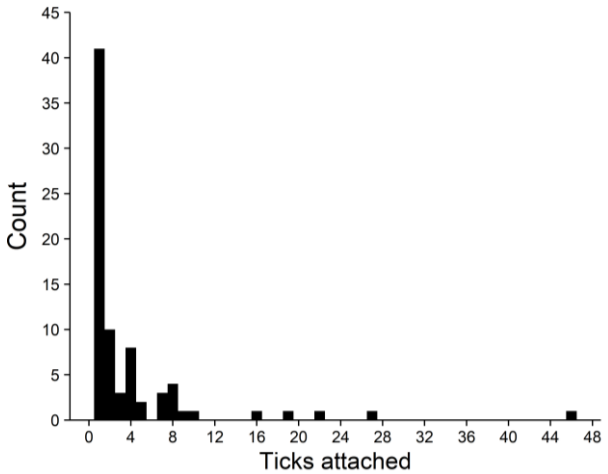


Table S1. Estimates and P-values of the effects of host traits (host haematocrit included), tick fasting time and feeding density on tick performance with number of birds in each model (N). No model selection was carried out. In bold, values with $P < 0.05$; "-" variable not included; "s." success.

	Host									
	BC		BC change		Sex		Age		Haematocrit	
Larvae	Est	*P*	Est	*P*	Est	*P*	Est	*P*	Est	*P*
On host										
Attachment s.	0.007	0.953	0.224	0.096	-0.025	0.886	-0.029	0.701	-0.010	0.910
Feeding time	-0.025	0.510	-0.004	0.927	-0.044	0.446	-0.029	0.236	-0.085	**0.004**
Weight	-0.516	0.158	0.927	**0.037**	1.256	**0.021**	0.231	0.331	0.581	**0.032**
Feeding s.	-0.045	0.746	0.024	0.884	0.644	**0.002**	-0.103	0.249	-0.217	**0.035**
Off host										
Moulting time	0.031	0.591	0.015	0.827	-0.007	0.930	-0.004	0.908	0.010	0.808
Moulting s.	-0.044	0.765	0.081	0.654	0.203	0.353	0.150	0.135	0.235	**0.026**
Overall survival	-0.071	0.511	0.048	0.702	0.511	**0.001**	-0.021	0.767	-0.022	0.780
Nymphs										
On host										
Attachment s.	-0.073	0.495	-0.076	0.445	-0.029	0.873	0.125	0.088	-0.239	**0.025**
Feeding time	0.077	0.253	-0.060	0.368	0.013	0.909	-0.084	0.077	0.031	0.544
Weight	-0.030	0.850	0.097	0.494	-0.487	0.064	0.131	0.200	-0.126	0.340
Feeding s.	0.045	0.765	0.111	0.395	-0.127	0.597	0.105	0.280	-0.138	0.257
Off host										
Moulting time	0.073	0.362	-0.044	0.594	0.155	0.241	-0.125	**0.030**	0.009	0.886
Moulting s.	0.166	0.642	-0.167	0.556	-0.497	0.396	0.691	**0.045**	-0.181	0.448
Overall survival	-0.008	0.939	0.000	0.996	-0.142	0.380	0.164	**0.009**	-0.195	**0.020**

	Tick			
	Fasting		Density	
Larvae	Est	*P*	Est	*P*
On host				
Attachment s.	0.108	0.250	-	-
Feeding time	0.107	**<0.001**	0.001	0.937
Weight	0.300	0.405	0.013	0.870
Feeding s.	0.078	0.493	0.005	0.868
Off host				
Moulting time	0.137	**0.009**	-0.006	0.628
Moulting s.	-0.302	0.100	0.007	0.828
Overall survival	-0.036	0.678	-	-
Nymphs				
On host				
Attachment s.	-0.204	0.055	-	-
Feeding time	0.031	0.602	0.057	**0.032**
Weight	-0.235	0.102	-0.056	0.452
Feeding s.	-0.106	0.403	-0.043	0.526
Off host				
Moulting time	0.218	**0.003**	0.083	**0.022**
Moulting s.	-0.118	0.643	-0.242	0.140
Overall survival	-0.143	0.099	-	-

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

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

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.

Table S2. Estimates and P-values of the effects of host traits (host haematocrit excluded), tick fasting time and feeding density on tick performance with number of infestations in each model (N). No model selection was carried out. In bold, values with $P < 0.05$; "-" variable not included; "s." success.

	Host							
	BC		BC change		Sex		Age	
	Est	*P*	Est	*P*	Est	*P*	Est	*P*
Larvae								
On host								
Attachment s.	-0.046	0.584	0.199	**0.035**	0.080	0.513	-0.028	0.652
Feeding time	-0.037	0.286	0.011	0.769	-0.056	0.267	-0.022	0.353
Weight	-0.406	0.220	0.542	0.111	0.740	0.118	0.254	0.263
Feeding s.	-0.025	0.787	0.097	0.337	0.507	**<0.001**	-0.032	0.655

Off host								
Moulting time	0.060	0.233	-0.024	0.649	-0.029	0.691	0.016	0.647
Moulting s.	0.014	0.910	0.096	0.448	0.006	0.973	0.159	0.074
Overall survival	-0.047	0.617	0.059	0.541	0.426	**0.002**	-0.008	0.896
Nymphs								
On host								
Attachment s.	-0.143	0.144	0.065	0.512	-0.074	0.663	0.138	0.079
Feeding time	0.032	0.601	0.023	0.716	0.040	0.705	-0.081	0.110
Weight	-0.127	0.391	0.071	0.614	-0.175	0.483	0.080	0.450
Feeding s.	0.080	0.520	0.112	0.464	-0.180	0.416	0.111	0.235
Off host								
Moulting time	0.031	0.642	0.040	0.538	0.183	0.108	-0.106	0.051
Moulting s.	0.206	0.487	-0.047	0.866	-0.168	0.743	0.811	**0.033**
Overall survival	-0.008	0.918	0.080	0.352	-0.163	0.248	0.201	**0.001**

	Tick			
	Fasting		Density	
	Est	*P*	Est	*P*
Larvae				
On host				
Attachment s.	0.129	0.102	-	-
Feeding time	0.120	**<0.001**	-0.001	0.881
Weight	-0.182	0.585	0.034	0.614
Feeding s.	-0.044	0.602	0.002	0.928
Off host				
Moulting time	0.137	**0.003**	-0.005	0.630
Moulting s.	-0.288	**0.035**	0.012	0.635
Overall survival	-0.090	0.311	-	-
Nymphs				
On host				
Attachment s.	-0.230	**0.017**	-	-
Feeding time	0.033	0.550	0.020	0.411
Weight	-0.137	0.308	0.008	0.900
Feeding s.	-0.145	0.268	0.020	0.722
Off host				
Moulting time	0.203	**0.001**	0.031	0.270
Moulting s.	-0.154	0.518	-0.049	0.693
Overall survival	-0.195	**0.013**	-	-

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

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

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.

Between-stage correlations in tick performance

On-host tick performance variables

Attachment success

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: Prop_attSuccLar | mi() ~ 1 + (1|BATCH.larvae)
Prop_attSuccNym | mi() ~ 1 + (1|BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 250000; warmup = 125000; thin = 100; total post-warmup samples = 5000

Feeding time

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: AvgFeedTime.larvae | mi() ~ 1 + (1 | BATCH.larvae)
AvgFeedTime.nymphs | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 220000; warmup = 110000; thin = 80; total post-warmup samples = 5500

Engorgement weight

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: AvgEngWgt.larvae | mi() ~ 1 + (1 | BATCH.larvae)
AvgEngWgt.nymphs | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 195000; warmup = 97500; thin = 70; total post-warmup samples = 5572

Feeding success

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: Prop_feedSuccLar | mi() ~ 1 + (1 | BATCH.larvae)
Prop_feedSuccNym | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 235000; warmup = 117500; thin = 90; total post-warmup samples = 5223

Table S3. Estimates with lower (l-95% CI) and upper (u-95% CI) 95% credible intervals of the (residual) correlations between larvae and nymphs in on-host tick performance variables. In bold, estimates not overlapping zero. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS)[1].

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Attachment success							
Group-level effects:							
Batch larvae	0.565	0.195	0.296	1.039	1.00	4887	4912
Batch nymphs	0.068	0.046	0.007	0.184	1.00	4751	4792
Population-level effects:							
Intercept larvae	0.067	0.203	-0.329	0.469	1.00	4866	5113
Intercept nymphs	0.694	0.038	0.611	0.761	1.00	4877	5008
Family-specific parameters:							
Sigma larvae	0.874	0.051	0.785	0.981	1.00	5166	4620
Sigma nymphs	0.150	0.012	0.130	0.175	1.00	4821	4950
Residual corr.:	**0.351**	0.155	**0.018**	**0.610**	1.00	4869	5122
Feeding time							
Group-level effects:							
Batch larvae	0.389	0.145	0.200	0.750	1.00	5450	5021
Batch nymphs	0.125	0.102	0.006	0.375	1.00	5272	5298
Population-level effects:							
Intercept larvae	0.570	0.148	0.276	0.867	1.00	5507	5416
Intercept nymphs	0.966	0.083	0.794	1.124	1.00	5592	5374
Family-specific parameters:							
Sigma larvae	0.374	0.025	0.329	0.427	1.00	5088	5302
Sigma nymphs	0.458	0.035	0.395	0.534	1.00	5358	5331
Residual corr.:	-0.320	0.186	-0.628	0.088	1.00	5443	5300
Engorgement weight							
Group-level effects:							
Batch larvae	0.941	0.322	0.518	1.739	1.00	5498	5016
Batch nymphs	0.237	0.209	0.009	0.768	1.00	5168	5088
Population-level effects:							
Intercept larvae	0.035	0.353	-0.673	0.769	1.00	5305	5263
Intercept nymphs	0.009	0.166	-0.302	0.360	1.00	5548	5532
Family-specific parameters:							
Sigma larvae	0.695	0.046	0.612	0.793	1.00	5504	5495
Sigma nymphs	1.024	0.079	0.882	1.191	1.00	5606	5341
Residual corr.:	0.440	0.264	-0.246	0.771	1.00	5577	5457
Feeding success							
Group-level effects:							
Batch larvae	0.100	0.033	0.052	0.180	1.00	5353	5277
Batch nymphs	0.096	0.057	0.028	0.238	1.00	4712	4350

Population-level effects:							
Intercept larvae	0.463	0.036	0.388	0.534	1.00	5210	4688
Intercept nymphs	0.716	0.049	0.611	0.810	1.00	5066	5148
Family-specific parameters:							
Sigma larvae	0.167	0.010	0.150	0.187	1.00	4918	4832
Sigma nymphs	0.163	0.013	0.140	0.190	1.00	5029	4994
Residual corr.:	0.087	0.153	-0.222	0.372	1.00	5368	5080

Residual corr.: residual correlations

Off-host tick performance variables

Moulting time

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: AvgMoultTimeNor.larvae | mi() ~ 1 + (1 | BATCH.larvae)
AvgMoultTimeNor.nymphs | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 150000; warmup = 75000; thin = 55; total post-warmup samples = 5455

Moulting success

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: Prop_moultSuccLar | mi() ~ 1 + (1 | BATCH.larvae)
Prop_moultSuccNym | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 180000; warmup = 90000; thin = 65; total post-warmup samples = 5539

Overall survival

Family: MV(gaussian, gaussian)
Links: mu = identity; sigma = identity
mu = identity; sigma = identity
Formula: Prop_survSuccLar | mi() ~ 1 + (1 | BATCH.larvae)
Prop_survSuccNym | mi() ~ 1 + (1 | BATCH.nymphs)
Data: RepeatabDataset (Number of observations: 230)
Samples: 4 chains, each with iter = 190000; warmup = 95000; thin = 70; total post-warmup samples = 5429

Table S4. Estimates with lower (l-95% CI) and upper (u-95% CI) 95% credible intervals of the (residual) correlations between larvae and nymphs in off-host tick performance variables and overall survival. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Moulting time							
Group-level effects:							
Batch larvae	0.332	0.126	0.171	0.656	1.00	4989	4896
Batch nymphs	0.148	0.114	0.007	0.422	1.00	5254	5021
Population-level effects:							
Intercept larvae	0.592	0.131	0.322	0.849	1.00	5219	5082
Intercept nymphs	0.573	0.095	0.385	0.762	1.00	4894	5208
Family-specific parameters:							
Sigma larvae	0.363	0.024	0.318	0.413	1.00	5375	5257
Sigma nymphs	0.483	0.038	0.417	0.564	1.00	5180	5030
Residual corr.:	-0.155	0.213	-0.543	0.271	1.00	5320	5453
Moulting success							
Group-level effects:							
Batch larvae	0.917	0.324	0.496	1.709	1.00	5218	5024
Batch nymphs	0.185	0.168	0.008	0.601	1.00	5267	5115
Population-level effects:							
Intercept larvae	0.053	0.343	-0.624	0.751	1.00	5615	5403
Intercept nymphs	-0.050	0.134	-0.289	0.248	1.00	5673	5127
Family-specific parameters:							
Sigma larvae	0.725	0.048	0.640	0.825	1.00	4872	5417
Sigma nymphs	0.774	0.060	0.666	0.899	1.00	5670	5421
Residual corr.:	-0.096	0.167	-0.411	0.238	1.00	5417	5522
Overall survival							
Group-level effects:							
Batch larvae	0.136	0.052	0.071	0.266	1.00	5474	5230
Batch nymphs	0.088	0.051	0.019	0.215	1.00	4910	4823
Population-level effects:							
Intercept larvae	0.334	0.054	0.225	0.438	1.00	5648	5174
Intercept nymphs	0.466	0.045	0.369	0.547	1.00	5253	5141
Family-specific parameters:							
Sigma larvae	0.141	0.009	0.123	0.161	1.00	5230	4948
Sigma nymphs	0.167	0.013	0.144	0.195	1.00	5525	5234
Residual corr.:	0.346	0.218	-0.139	0.681	1.00	5460	5438

Animal models

On-host tick performance variables

Attachment success

Larvae: brm(TICK_ATT|trials(TICKS_total) ~ 1 + (1|gr(animal, cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = binomial(), chains = 4, iter = 190000, warmup = 95000, thin = 45, total post-warmup samples = 8445).

Nymphs: brm(TICK_ATT|trials(TICKS_total) ~ 1 +(1|gr(animal, cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 170000, warmup = 85000, thin = 40, total post-warmup samples = 8500).

Table S5. Output animal model on attachment success of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk	Tail
Larvae							
Group-level effects:							
sd(Animal)	0.495	0.069	0.365	0.635	1.00	8118	8102
sd(Batch)	0.507	0.176	0.263	0.925	1.00	8121	8066
Population-level effects:							
Intercept	2.852	0.183	2.488	3.219	1.00	8427	7888
Nymphs							
Group-level effects:							
sd(Animal)	0.358	0.142	0.060	0.621	1.00	8376	8096
sd(Batch)	0.341	0.211	0.041	0.855	1.00	8041	7968
Population-level effects:							
Intercept	0.850	0.187	0.448	1.198	1.00	8362	8356

Feeding time

Larvae: brm(FeedTimeNor ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = gaussian(), chains = 4, iter = 1300000, warmup = 650000, thin = 350, total post-warmup samples = 7429).

Nymphs: brm(FeedTimeNor ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = gaussian(), chains = 4, iter = 380000, warmup = 190000, thin = 90, total post-warmup samples = 8445).

Table S6. Output animal model on feeding time of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	0.341	0.065	0.164	0.424	1.00	6108	7010
sd(Batch)	0.336	0.132	0.171	0.667	1.00	7132	7224
Population-level effects:							
Intercept	0.558	0.135	0.286	0.826	1.00	7134	6846
Family-specific parameters:							
Sigma	0.134	0.089	0.013	0.339	1.00	4736	3969
Nymphs							
Group-level effects:							
sd(Animal)	0.065	0.037	0.004	0.136	1.00	8151	8178
sd(Batch)	0.052	0.043	0.002	0.162	1.00	8454	8697
Population-level effects:							
Intercept	1.555	0.034	1.483	1.618	1.00	8545	8344
Family-specific parameters:							
Sigma	0.151	0.019	0.111	0.187	1.00	8425	8287

Engorgement weight

Larvae: brm(AvgEngWgt ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = gaussian(), chains = 4, iter = 710000, warmup = 400000, thin = 150, total post-warmup samples = 8267).

Nymphs: brm(AvgEngWgt ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = gaussian(), chains = 4, iter = 340000, warmup = 170000, thin = 80, total post-warmup samples = 8500).

Table S7. Output animal model on engorgement weight of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	1.002	0.632	0.051	2.272	1.00	8357	8018
sd(Batch)	3.014	0.962	1.720	5.425	1.00	8271	7639
Population-level effects:							
Intercept	26.125	1.094	23.967	28.339	1.00	8194	7892

Family-specific parameters:							
Sigma	2.031	0.392	1.012	2.575	1.00	8148	7562
Nymphs							
Group-level effects:							
sd(Animal)	6.975	4.504	0.322	16.420	1.00	8250	8491
sd(Batch)	4.131	3.544	0.151	13.250	1.00	7556	7763
Population-level effects:							
Intercept	218.295	3.293	211.846	225.109	1.00	8518	8341
Family-specific parameters:							
Sigma	20.726	2.262	15.960	24.964	1.00	8514	8393

Feeding success

Larvae: brm(Eng_lar|trials(TICK_ATT) ~ 1 + (1|gr(animal, cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 105000, warmup = 52500, thin = 25, total post-warmup samples = 8400).

Nymphs: brm(Eng_nym|trials(TICK_ATT) ~ 1 + (1|gr(animal, cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 130000, warmup = 65000, thin = 30, total post-warmup samples = 8667).

Table S8. Output animal model on feeding success of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	0.818	0.060	0.705	0.943	1.00	8504	8563
sd(Batch)	0.401	0.145	0.188	0.756	1.00	8276	8288
Population-level effects:							
Intercept	-0.150	0.156	-0.469	0.161	1.00	8309	7767
Nymphs							
Group-level effects:							
sd(Animal)	0.345	0.168	0.031	0.667	1.00	8597	8669
sd(Batch)	0.507	0.275	0.144	1.196	1.00	8220	8209
Population-level effects:							
Intercept	0.988	0.256	0.441	1.460	1.00	8724	8463

Off-host tick performance variables

Moulting time

Larvae: brm(MoultTimeNor ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = gaussian(), chains = 4, iter = 160000, warmup = 80000, thin = 40, total post-warmup samples = 8000).

Nymphs: brm(MoultTimeNor ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = gaussian(), chains = 4, iter = 255000, warmup = 127500, thin = 60, total post-warmup samples = 8500).

Table S9. Output animal model on moulting time of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	0.097	0.069	0.004	0.254	1.00	7916	7559
sd(Batch)	0.339	0.133	0.170	0.674	1.00	7163	7689
Population-level effects:							
Intercept	0.587	0.134	0.323	0.852	1.00	8010	7757
Family-specific parameters:							
Sigma	0.345	0.033	0.271	0.406	1.00	7998	7849
Nymphs							
Group-level effects:							
sd(Animal)	0.148	0.104	0.005	0.380	1.00	8297	8226
sd(Batch)	0.139	0.111	0.007	0.421	1.00	8178	7863
Population-level effects:							
Intercept	0.575	0.092	0.383	0.754	1.00	8459	7891
Family-specific parameters:							
Sigma	0.455	0.054	0.331	0.551	1.00	8298	8155

Moulting success

Larvae: brm(Moulted_lar|trials(Eng_lar) ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 143000, warmup = 71500, thin = 30, total post-warmup samples = 9534).

Nymphs: brm(Moulted_nym|trials(Eng_nym) ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 150000, thin = 35, warmup = 75000, total post-warmup samples = 8572).

Table S10. Output animal model on moulting success of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	0.756	0.079	0.615	0.924	1.00	9279	9241
sd(Batch)	1.294	0.436	0.715	2.389	1.00	9299	9028
Population-level effects:							
Intercept	1.216	0.484	0.214	2.153	1.00	8567	9160
Nymphs							
Group-level effects:							
sd(Animal)	1.219	0.366	0.563	2.011	1.00	8257	8274
sd(Batch)	0.821	0.768	0.033	2.735	1.00	8459	8085
Population-level effects:							
Intercept	3.089	0.603	2.123	4.423	1.00	8348	8309

Overall survival

Larvae: brm(Moulted_lar|trials(TICKS_total) ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Larvae, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 140000, warmup = 70000, thin = 30, total post-warmup samples = 9334).

Nymphs: brm(Moulted_nym|trials(TICKS_total) ~ 1 + (1|gr(animal,cov = A)) + (1|BATCH), data = Nymphs, data2 = list(A = A), family = binomial(link = "logit"), chains = 4, iter = 150000, warmup = 75000, thin = 35, total post-warmup samples = 8572).

Table S11. Output animal model on overall survival of larvae and nymphs. Lower (l-95% CI) and upper (u-95% CI) 95% credible intervals. On the right, the potential scale reduction factor on split chains (Rhat; 1 at convergence between chains), the effective sample size based on the rank normalized draws (Bulk ESS), and minimum effective sample sizes of the 5% and 95% quantiles (Tail ESS).

	Estimate	Est. Error	l-95% CI	u-95% CI	Rhat	Bulk ESS	Tail ESS
Larvae							
Group-level effects:							
sd(Animal)	0.669	0.058	0.564	0.793	1.00	9318	9335
sd(Batch)	0.712	0.259	0.378	1.359	1.00	8818	9213
Population-level effects:							
Intercept	-0.774	0.280	-1.334	-0.202	1.00	8872	9180
Nymphs							
Group-level effects:							
sd(Animal)	0.290	0.122	0.036	0.513	1.00	8487	8602
sd(Batch)	0.409	0.214	0.142	0.950	1.00	8368	8451
Population-level effects:							
Intercept	-0.159	0.201	-0.592	0.225	1.00	8336	8279

Fig. S4. Posterior distribution of heritability estimates for success ratios in larvae and nymphs. The vertical line represents the mode of the distribution.

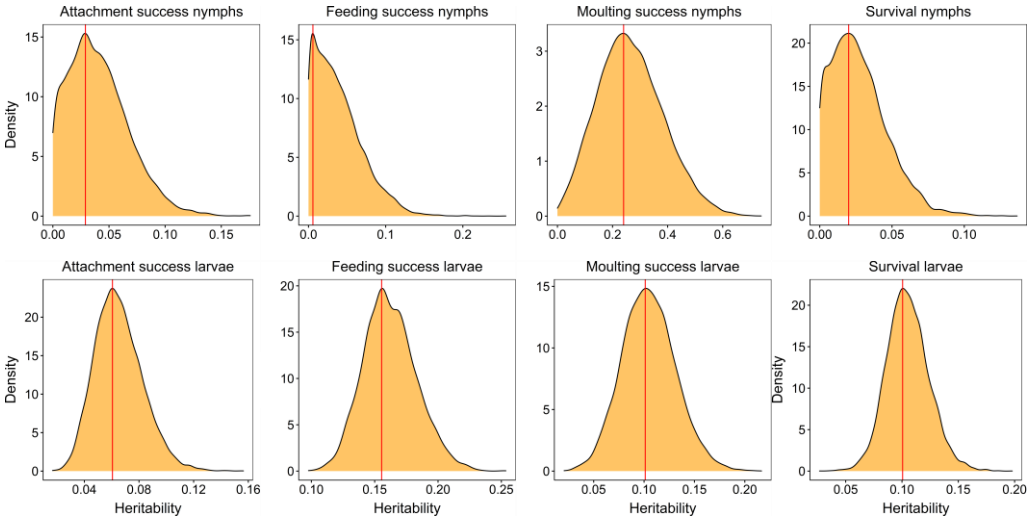


Fig. S5. Posterior distribution of heritability estimates for feeding time, engorgement weight, and moulting time in larvae and nymphs. The vertical line represents the mode of the posterior distribution.

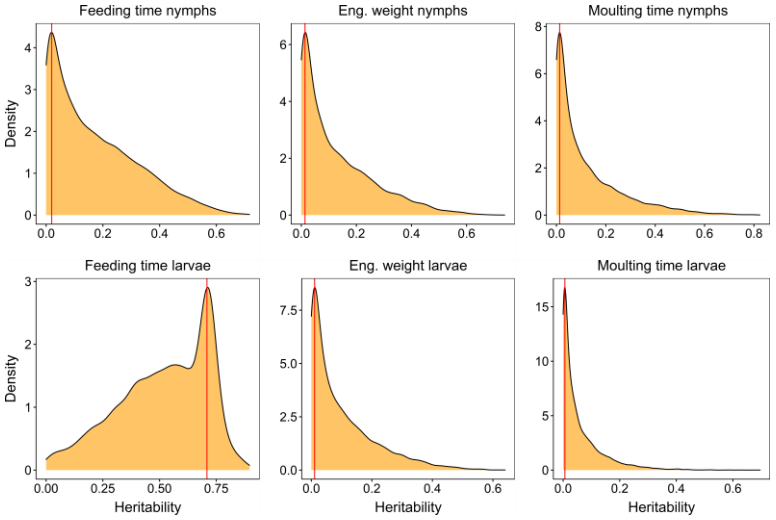


Table S12. Mean additive genetic variance (V_a) and residual variance (V_r) for all tick performance variables.

Variable	Additive genetic variance		Residual variance	
	Larvae	Nymphs	Larvae	Nymphs
On host				
Attachment success	0.250	0.148	3.577	3.450
Feeding time	0.121	0.006	0.156	0.028
Engorgement weight	1.403	68.940	14.288	464.318
Feeding success	0.673	0.147	3.471	3.623
Off host				
Moulting time	0.014	0.033	0.253	0.241
Moulting success	0.578	1.620	5.154	4.554
Overall survival	0.451	0.099	3.863	3.503

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