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

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Little is known about the intraspecific variation of parasite life-history traits and 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 molting success. Molting 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 molting time, the latter supported by a negative genetic correlation. We found sex-related differences in feeding time (longer in male nymphs) and molting time (longer in male larvae but shorter in male nymphs). Also, time since the last feeding event (set experimentally) reduced larval and nymphal fitness, whereas it increased adult female fitness. Furthermore, we found significant heritability and evolvability, that is, the potential to respond to selection, for engorgement weight and molting 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.

KEY WORDS: Evolutionary potential, heritability, host-parasite interactions, *Ixodes arboricola*, parasite success, *Parus major*.

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 counteradaptations (Clayton and Moore 1997; Sorci et al. 1997; Carius et al. 2001; Poulin 2007). Comprehending how parasite life-history traits covary, their relative contribution to parasite fitness and evolutionary potential will show us how parasites may adapt to new selection pressures and evolve different life-history strategies (Clayton and Moore 1997; Barrett et al. 2008; Clayton et al. 2015), providing a crucial basis for the development of predictive tools for disease monitoring and prevention (Levin et al. 1999; Dronamraju 2004; Tolle 2009; Anderson et al. 2010; Dantas-Torres et al. 2012).

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 (Kruuk et al. 2001; Morrissey et al. 2012; Aguirre et al. 2014; 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. To optimize their fitness, parasites are expected to evolve traits that facilitate host

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exploitation and transmission without necessarily leading to higher virulence, that is, damage to the host (Clayton and Tompkins 1994; Poulin 2007; Alizon et al. 2009; Heylen and Matthysen 2011a). 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 (Poulin 2007; Mazé-Guilmo et al. 2014).

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. Although many studies have examined the heritability and (micro)evolution of host traits related to resistance or tolerance (Boulinier et al. 1997; Hill 1998; Williamson and Kumar 2006; Kause et al. 2012; Mazé-Guilmo et al. 2014; Ayres et al. 2015), 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 (Madder et al. 1996; Li et al. 2005; Lysyk 2010). 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, because 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 behavior 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 (Jia et al. 2002; Nachappa et al. 2010; Durkin and Luong 2018; Durkin and Luong 2019) and infectious behavior of ticks (Young et al. 1995; Madder et al. 1996; Li et al. 2005; Lysyk 2010; Fragoso-Sanchez et al. 2011) but we are not aware of any studies on individual traits and how they correlate with fitness. Obligate but nonpermanent 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., molting), and reproduction (Van Oosten et al. 2016a; Van Oosten et al. 2018).

Ticks are common hematophagous ectoparasites of terrestrial vertebrates, and after mosquitoes are considered the second main vector of diseases for humans and farm animals (Parola and Raoult 2001; Dantas-Torres et al. 2012). 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 (McMeniman et al. 2014; Tomás and Soler 2016; Carr and Salgado 2019). Second, as observed in other ectoparasites, they need to overcome host behavioral (Clayton et al. 2010) and physiological defenses (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 defenses (Bize et al. 2008; Reid et al. 2014). Third, off-host ectoparasites have to survive and molt 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 (Gray 1981; Chen et al. 2009; Ma et al. 2013; Ginsberg et al. 2016; 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 (Li et al. 2005; Fragoso-Sanchez et al. 2011), 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, molting 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 genetically adaptive variation (Houle 1992; Hansen et al. 2011). 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 and 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. 2014b; Van Oosten et al. 2014b). This tick is an obligate nest-dwelling ectoparasite feeding on birds roosting or breeding in natural cavities (White et al. 2012; Heylen et al. 2014b; Van Oosten et al. 2014b). As most ixodid ticks, *I. arboricola* feeds once as larva and nymph before molting, with adult females, but not adult males, feeding a third time before egg laying (Sonenshine and Roe 2013b).

Great tits are small passerine birds (16–21 g) inhabiting woodlands, parks, and gardens throughout Europe, part of Asia and North Africa (Cramp and Perrins 1993). All birds came from a free-living population in the Boshoek area (51° 7′ 59″ N, 4° 31′ 1″ E) near Antwerp, Belgium (Matthysen 2002; Korsten et al. 2013). 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; Literak et al. 2007; Heylen et al. 2014b) as well as *Ixodes ricinus* (Hubalek et al. 1996; Heylen et al. 2013a) and more rarely with *Ixodes frontalis* (Heylen et al. 2013b, 2014a).

GENERAL STUDY DESIGN, TRAIT, AND SUCCESS VARIABLES

Between 2017 and 2019, two consecutive tick generations were raised in seminatural 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), whereas 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, whereas immature stages can feed on both adults and nestlings (Heylen et al. 2014b). In this way, we made optimal use of the limited temporal availability of wild great tit nestlings. Nymphs and adults were marked individually, whereas unfed larvae were only identified at the clutch level (protocol details below).

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) molting 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 defense (e.g., grooming and immune defense). 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 (Jones and Nuttall 1990;

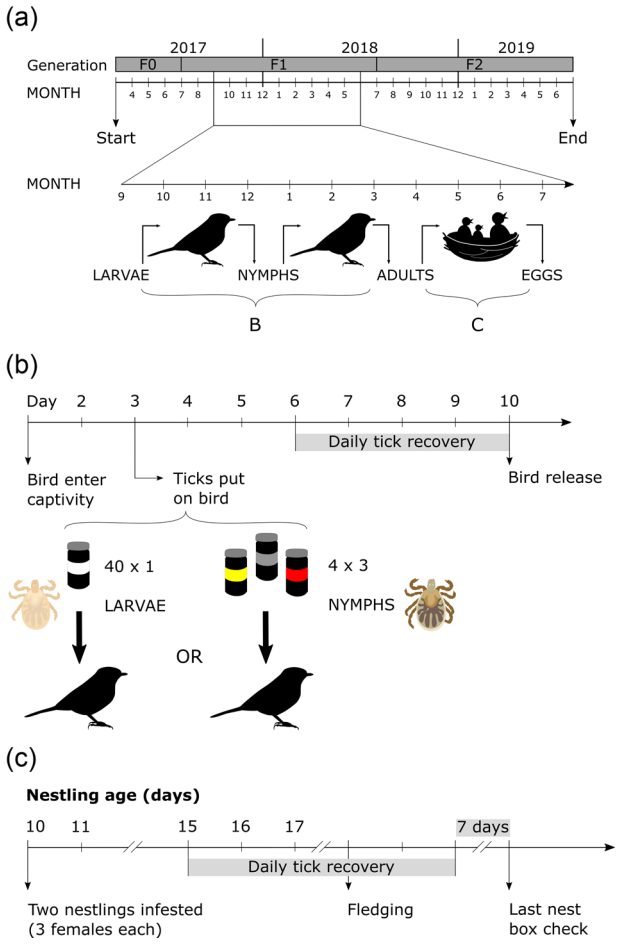

Figure 1. Study design. (a) Overview across years. Adult ticks were collected in the wild (F0) and bred for two generations (F1, F2) starting in March 2017. Within each generation, larvae and nymphs were fed on adult great tits between October and March, and on nestlings during the breeding season. (b) Overview of infestation procedure for ticks put on adult great tits in lab conditions. After 2 days of acclimatization, every bird was infested with 65 larvae from one clutch or 12 nymphs from three clutches (four 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.

Gebbia et al. 1995). 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. Molting 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 molt quickly can feed a second time within the period of host availability and gain a fitness advantage (Heylen et al. 2012, 2014b). However, ticks with higher engorgement weights may also need a longer molting 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 molting 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 molting time (called "premolting period" in some studies) as the number of days elapsed between collection and emergence from the 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 semitransparent vials hindering larval counts), whereas 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, molting 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 due to their change in shape and size. We did not recover any living ticks that had not engorged (see also Supporting Information, SI hereinafter). Molting success (larvae and nymphs) was defined as the successful molting once a tick was engorged. Survival success describes the survival of an infested tick until successful molting (larvae and nymphs) or egg hatching (adult females) thus encompassing feeding and molting success. Finally, egg-laying success (adult females) was defined as laying at least one egg (see Table 1 for an overview of the variables).

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, because 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 4 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 9 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. Because *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). Because 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 standardized artificial daylight (10 h 30 min including dawn and sunset), 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 acclimatize 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 and Matthysen 2010; Heylen et al. 2014a, 2017) and in accordance with natural attachment behavior (Fracasso

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

Variable	Description	Life stage
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
Molting time	Days elapsed between recovery and completion of molting.	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
Molting 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 (within stage).	F
Fasting time	Days elapsed between recovery or hatching and the next infestation.	L, N, F
Batch	Period when a group of birds was simultaneously infested.	L, N
Feeding event	ID of infestation (bird × batch combination; nest ID for adult females).	L, N, F

et al. 2019). Every bird was infested with either approximately 65 larvae, or 12 nymphs with four nymphs from each of three different clutches. Because 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 behavior or performance between clipped and unclipped ticks (results not shown). To prevent impairment in host finding behavior, the first pair of limbs holding the Haller's organ was not clipped (Sonenshine and Roe 2013a; Romanenko et al. 2016). Immediately after infestation, birds were put in an air-permeable cotton bag for 1 h (Heylen et al. 2014a, 2017; Fracasso et al. 2019). 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 5 days, and engorged ticks were collected (Fig. 1b). 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 because they could have detached at the earliest by the next morning due to the natural detachment behavior 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 three 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 reused 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. Because very few F0 females were recovered at 4 days, nest boxes for F1 and F2 were checked daily from the fifth day until recovery of all engorged females, or until 2 days after fledging (Fig. 1c). As for adult F0 females, the nest box was checked one last time 9 days after fledging if not all ticks had previously been recovered. Females recovered after day eight were assigned an unknown feeding time because they likely had detached earlier but remained temporarily hidden in the nest material. After engorgement, F1 and F2 adult females were mated with one randomly chosen nonsibling 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 8 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 molting, egg laying, or egg hatching except for a few occasions where checks occurred with 2- or 3-day intervals.

We started the F1 generation with larvae from 51 clutches (F0), and the F2 generation with 48 clutches. Per generation, we obtained 1600–1800 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). The models predicting attachment success, feeding success, and survival included 1349 and 304 unfed nymphs and adult females, respectively. Analyses of molting 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, molting, survival, and egg-laying success were analyzed at the individual tick level by fitting separate Bayesian generalized linear mixed models with a Bernoulli distribution using the "brms" (version 2.15.0) package (Bürkner 2017, 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, because 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 2–3 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," whereas 95% confidence intervals (see below) will be written in full.

Sources of genetic and environmental variation

Variation in tick traits was analyzed by fitting a trivariate Animal Model (generalized linear mixed model) in a Bayesian framework (Kruuk et al. 2008; Wilson et al. 2010; Brommer et al. 2019; de Villemereuil 2019) using the "MCMCglmm" (version 2.32) package (Hadfield 2010, 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, molting time (larvae and nymphs), and number of hatched eggs (adult females). Because molting 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. Because 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 nonheritable components, such as behavioral and physiological defenses) 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 nonsurviving 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) because 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" (version 0.19.3) and "MCMCvis" (version 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 (Houle 1992; Wilson 2008; Hansen et al. 2011), we also calculated the coefficient of additive genetic variation (CV_a), the coefficient of residual variation (CV_r), and the mean-standardized additive variance (I_a) following Houle (1992). Because 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 (Houle et al. 2011; Garcia-Gonzalez et al. 2012). 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" (version 0.7.4) package (de Villemereuil et al. 2016; de Villemereuil 2020) and used to calculate heritability on the observed scale (h^2_o), 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 standardized and normalized data using Kendall's tau correlation with 95% confidence intervals (10^4 bootstrap iterations) using the "NSM3" (version 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 molting 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), whereas molting 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 molted 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 molting 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 Tables S1 and S2).

PREDICTORS OF FITNESS

Higher attachment success (adult females) was correlated with longer molting time to the adult stage (Tables 2 and 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 molting time, whereas no such association was found in nymphs. Molting 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 molting success in larvae, whereas it positively affected feeding success and survival in adult females.

Table 2. Predictors of tick success (fixed effects) in Bayesian generalized linear mixed models: means and 95% credible intervals (squared brackets).

Success trait	Feeding time	Engorgement weight	Molting 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]**
Molting				
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]		

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 Supporting Information.

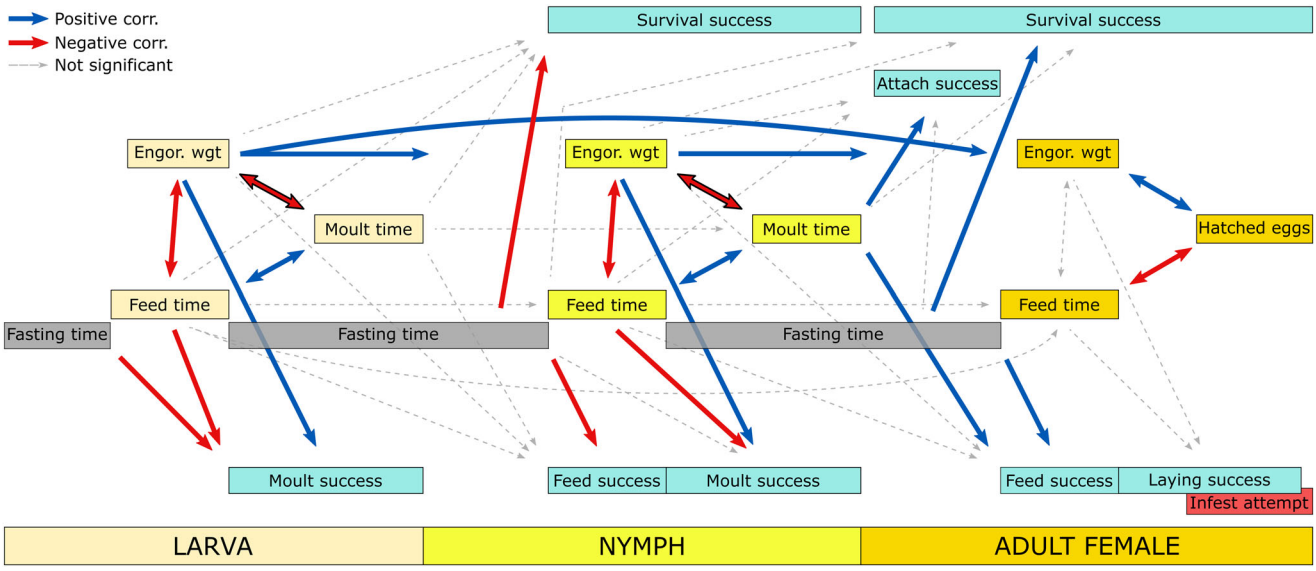

Figure 2. Overview of the main findings for predictors of tick success and correlations between traits. Phenotypic correlations are shown as double-sided arrows and have a black outline if supported by a significant genetic correlation. Single-sided arrows show the effect of predictors on success parameters (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 (gray dashed arrows).

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 molting success of larvae.

PHENOTYPIC CORRELATIONS AMONG TRAITS

At the phenotypic level (Table 3), engorgement weight was negatively correlated with feeding time (Fig. 3) and molting time in larvae and nymphs, whereas feeding time increased with molting time (Fig. S3). In adult females, the number of hatched eggs increased with engorgement weight, whereas 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 (τ = 0.460; Table S8; Fig. S4), whereas there were

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.

	Feeding time	Engorgement weight	Molting time/hatched eggs[1]
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)[1]
Engorgement 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)[1]
Molting time			
Larvae	0.390 [−0.411, 0.993]	–0.643 [−0.978, −0.229][1]	
Nymphs	0.339 [−0.366, 0.944]	–0.389 [−0.823, −0.008][1]	
Hatched eggs			
Adult females	–0.099 [−0.877, 0.752]	0.410 [−0.351, 0.971]	

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 untransformed data).

[1] Molting time replaced by "Hatched eggs" for adult female ticks.

*** $P < 0.05$.**

**** $P < 0.001$.**

no across-stage correlations for feeding time or molting time.

EVOLUTIONARY POTENTIAL AND GENETIC CORRELATIONS

Mean heritability and 95% CI for feeding time, engorgement weight, molting time (larvae and nymphs), and number of hatched eggs (adult females) are shown in Table 4 along with h^2_o, CV_a, CV_r, and I_a (nymphs). Because models on larvae with and without the sex effect led to highly similar results (Tables S9 and S10; Figs. S5 and S6), only the model with sex effect is reported below. In larvae, heritability is highest for molting 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 molting 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 molting time (Fig. S9). On the contrary, heritability for feeding time and number of hatched eggs may not be reliable. Although heritability of nymph engorgement weight was slightly higher than for molting time, molting 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 molting 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.

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), although with wide 95% CI, whereas 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, although the latter estimate had very wide credible intervals. Low, but significant, effects of feeding event were also found for engorgement weight and molting 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 molting time in nymphs (17.4%).

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 molting

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	Engorgement weight	Molting 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]	
Adult 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]	
Adult females	0.005 [−0.016; 0.025]	0.003 [−0.015; 0.021]		0.007 [−0.012; 0.025]
Year: 2018				
Adult females	–0.12 [−0.60; 0.35]	**0.80 [0.45; 1.17]**		1.05 [−0.38; 2.43]
Year: 2019				
Adult 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]	
Adult 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]	
Adult 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]	
Adult females[1]	0.136 [0.000; 0.362]	0.048 [0.000; 0.167]		0.067 [0.000; 0.227]
Nest identity				
Adult 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]	
Adult 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]	
Adult females	0.298 [0.000; 0.618]	0.597 [0.187; 0.955]		0.308 [0.000; 0.690]

Feeding time and molting 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.

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

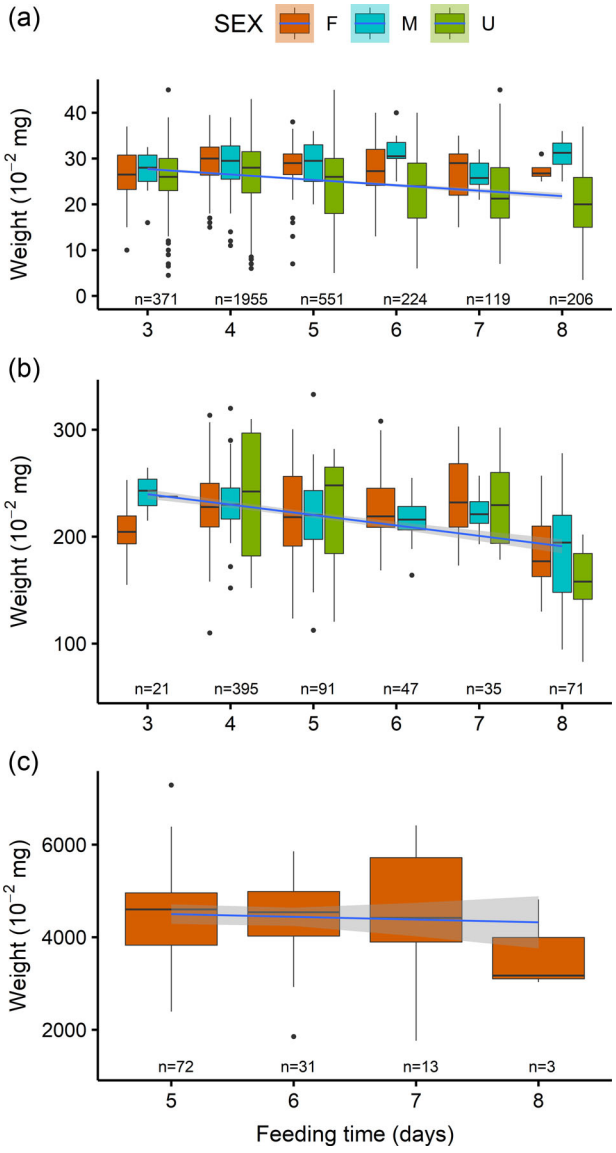

Figure 3. Phenotypic correlation between feeding time and engorgement weight for larvae (a), nymphs (b), and adult females (c). The blue line represents the linear regression fitted to these data with 95% confidence intervals in gray. Females (F) are shown in red, males (M) in light blue, and ticks of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

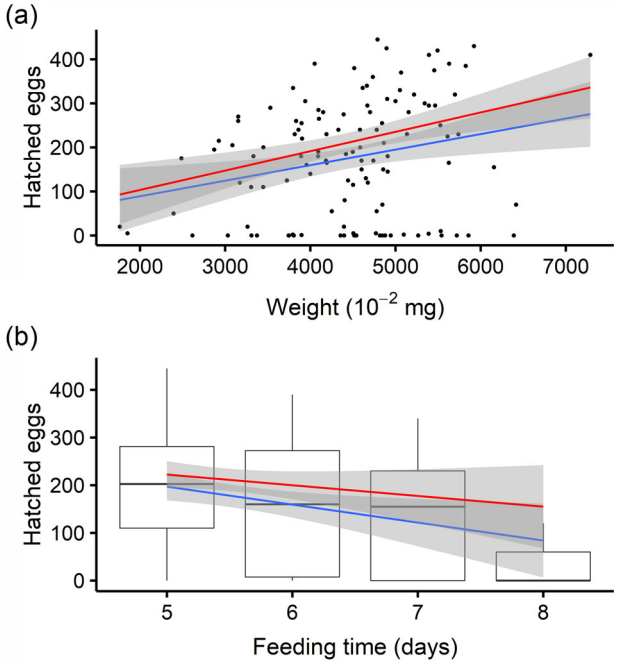

Figure 4. Correlation between number of hatched eggs and engorgement weight (a) and feeding time (b). Blue lines represent the linear regression with adult females with zero eggs included or excluded (red line) and 95% confidence intervals in gray.

times than females, but in nymphs this difference was reversed. Also, males had longer feeding times in nymphs, whereas 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 carryover 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 molting 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* metapopulation 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 because 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

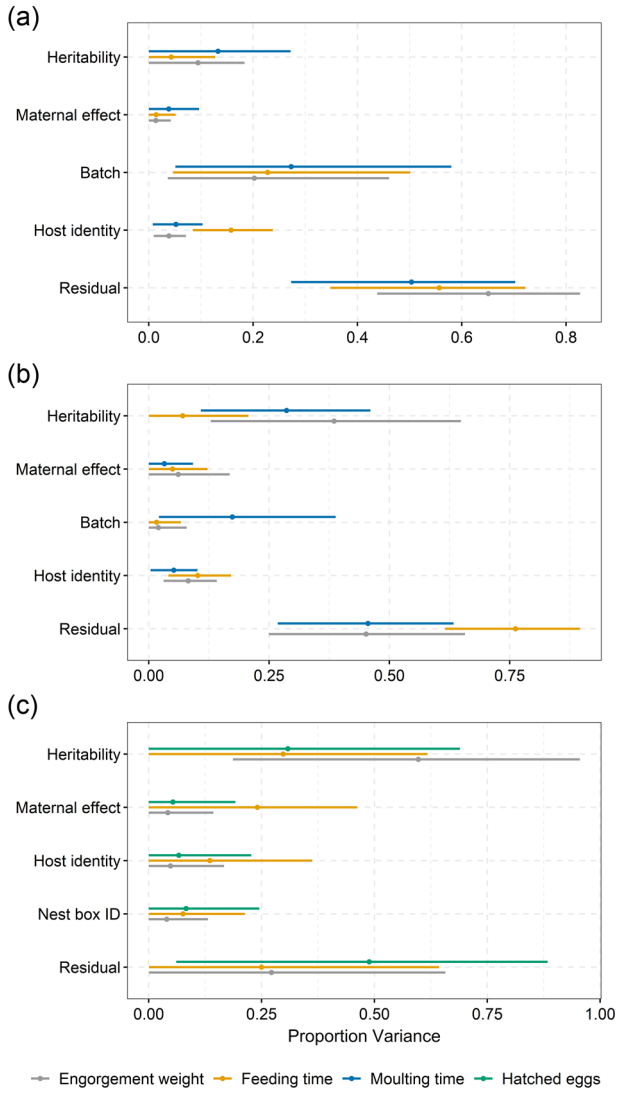

Figure 5. Posterior means and 95% credible intervals of heritability and other components of variation, expressed as proportions of total phenotypic variance after accounting for fixed effects. Panels represent output for larvae (a), nymphs (b), and adult females (c).

nymphs took longer to molt to adults. Interestingly, there was no effect of larval molting time on nymph feeding success suggesting stage-specific differences between nymphs and adults. On the other hand, larval and nymph molting 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 or 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 molt 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 defenses. 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, whereas 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 that 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 behavior 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 where differences between feeding sites are probably less pronounced. 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 antitick immunological resistance in our host (Heylen et al. 2010; Heylen et al. 2021). The hypothesis that engorgement weight reflects individual quality is confirmed when looking at molting time: intuitively, engorgement weight and molting time should be positively correlated because a bigger blood meal should take more time to be processed, as observed across stages (Heylen et al. 2014b). Instead, we found that within the same stage, ticks with higher engorgement weights and shorter feeding times needed less time to molt. 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 (Gray 1981; Ma et al. 2013; Ginsberg et al. 2016). Although 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. Although 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 because 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 molting 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 molting 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 molting 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, although 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 carryover effects later in life.

To our knowledge, this is one of the first studies showing heritable variation in a measure of host exploitation (Madder et al. 1996). In fact, engorgement weight and molting time show substantial estimates of evolvability and heritability across the three stages, and thus have the potential to evolve rapidly under changing selective pressures. Although longer molting 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 that 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) in 151 studies of animal populations (Moore et al. 2019). However, in our setup maternal effects may have been lower compared to natural conditions due to the standardized feeding of adult ticks on nestlings of the same species, similar age, and body 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 the expression of parasite traits, a fundamental requirement for host-parasite coevolution (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, that is, between birds in the number of ticks that actually attached (Wang et al. 2001; Bartosik and Buczek 2012; Van Oosten et al. 2016b). Surprisingly, batch effects were considerable despite highly standardized conditions. These may be attributed to between-batch differences in host physiology and behavior that affected tick parameters as in earlier studies (Tschirren et al. 2007; Bize et al.

2008; Seppälä et al. 2008). 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, whereas adult females mostly feed in the nestling period (Heylen et al. 2014b). 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 molting time. In particular, male larvae molted more slowly, whereas male nymphs fed for longer and molted 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 molting 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 parameters being 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) that 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 molting 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, whereas 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; Newson et al. 1984; Chilton and Bull 1993; Tsunoda 2008; Rosendale et al. 2017). 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 carryover effects can both account for the variation in tick quality. Furthermore, we show that key life-history traits such as engorgement weight and molting 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 underexploited tools to investigate the selective pressures and evolutionary potential of parasite traits. Here, we use this approach and show how nonpermanent 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.

AUTHOR CONTRIBUTIONS

EM and DH conceptualized the study and acquired the funding. EM, DH, and GF discussed the experimental design with data collection carried out by GF and JE. GF did the statistical analyses with support from SVD and EM. GF wrote the original draft. All authors approved the final version.

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DATA ARCHIVING

The data used in this study are publicly available in Dryad (https://doi.org/10.5061/dryad.37pvmcvm6).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS

Experiments were carried out under license of the Flemish Ministry (Agentschap Natuur en Bos; license: ANB-VL-FF-V17-00029) and the experimental protocol was approved by the Ethical Committee of the University of Antwerp, Belgium (no. 2016-88) in compliance with the Directive 2010/63/EU.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Number of clutches, engorged ticks, feeding and molting success, and number of infested hosts for the two consecutive tick generations (F1, F2) and their founders (F0)