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Distinct life history strategies underpin clear patterns of succession in microparasite communities infecting a wild mammalian host

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Abstract

Individual animals in natural populations tend to host diverse parasite species concurrently over their lifetimes. In free-living ecological communities, organismal life histories shape interactions with their environment, which ultimately forms the basis of ecological succession. However, the structure and dynamics of mammalian parasite communities have not been contextualized in terms of primary ecological succession, in part because few datasets track occupancy and abundance of multiple parasites in wild hosts starting at birth. Here, we studied community dynamics of twelve subtypes of protozoan microparasites (*Theileria* spp.) in a herd of African buffalo. We show that *Theileria* communities followed predictable patterns of succession underpinned by four different parasite life-history strategies. In contrast to many free-living communities, network complexity decreased with host age. Examining parasite communities through the lens of succession may better inform the effect of complex within host eco-evolutionary dynamics on infection outcomes, including parasite co-existence through the lifetime of the host.

Introduction

Ecological communities are shaped, at the most fundamental level, by succession of species as they colonize new habitats and subsequently interact with their environment to persist. As such, in the absence of major stochastic extirpation events, communities may follow temporally predictable patterns according to the events that initiate a new habitat patch and the component species' life histories (Leibold & Chase, Jonathan M., 2017; Miller & terHorst, 2012; Noble, IR & Slatyer, 1977; Pickett et al., 1987).

Classical succession theory states that communities increase in diversity through early successional stages, peak at mid-successional stages, and, if the community reaches equilibrium, slightly decline to a stable plateau at late successional stages (Miller & terHorst, 2012). Mechanistically, patterns of succession are hypothesized to be driven by habitat patch resource availability and species interaction networks. For example, classical ecological theory suggests that species in early successional stages often have life history strategies conducive to high dispersal rates so they can establish in new habitat patches before being displaced by slower dispersing, superior competitors (Tilman, 1994). Early communities may consist of positive and negative interactions, where pioneer species modify the environment to be more or less hospitable for later-colonizing species (Malkinson et al., 2003). In many free-living communities, the resources governing species interactions decrease as species richness increases (e.g., light for plants) leading to equilibrium communities with a higher proportion of negative interactions than early successional communities (Tilman, 1985). However, perturbations at early stages may prohibit communities from reaching a stable equilibrium state or drive communities towards alternative stable states (Connell, 1978; Kröel-Dulay et al., 2015; Lewontin, 1969). Altogether, mechanisms driving patterns of succession are key to understanding and predicting changes in species assemblages over time.

In parasite communities, early research suggested parasite interactions to be rare, and community structure to conform to neutral assembly models (Poulin, 2004). Since then, an abundance of work has pointed towards the importance of order of infection and parasite interaction dynamics on parasite community assembly and infection outcome (Budischak et al., 2016; Devevey et al., 2015;

Ezenwa & Jolles, 2015; Halliday et al., 2020; Johnson & Hoverman, 2012; Lello et al., 2004; Rynkiewicz et al., 2019; Telfer et al., 2010).

However, few studies have contextualized the structure and dynamics of parasite communities in terms of ecological succession, especially primary succession, in part because few datasets track occupancy and abundance of multiple parasites in wild hosts starting at birth (but see Budischak et al., 2016; Combrink et al., 2020; Espínola-Novelo et al., 2020; Mordecai et al., 2016) for succession based studies in wild systems). Life history trade-offs, such as the commonly observed negative association between dispersal and competitive ability, may play a role in structuring successional processes in parasite communities as they do in free-living systems. However, parasite communities differ from free-living communities in at least two important ways that may affect successional patterns: First, resource availability, especially in terms of space and nutrients, may increase, rather than decrease over time, because hosts (habitat patches) grow as they mature, perhaps resulting in a reduction in negative interactions in later successional communities (Griffiths et al., 2014; Rynkiewicz et al., 2015). Second, hosts, unlike abiotic habitat patches, can directly respond to and impact colonization and persistence through their immune responses (Rynkiewicz et al., 2015). This is likely to affect successional processes in novel ways. For example, negative intra-specific interactions may increase and inter-specific interactions may decrease with animal age as the adaptive immune system develops (i.e., the immune system more precisely targets the focal pathogen species); as such colonization patterns may emerge from interactions with both co-infecting parasites and the host immune system, with the relative impact of each interaction changing through time. Further, infection by immunosuppressive parasites may facilitate the colonization of different parasites and trigger community development through

positive interactions as opposed to negative (Ezenwa & Jolles, 2015; Johnson et al., 2015; Selik et al., 1984). Thus, while many aspects of parasite community ecology mirror dynamics observed in free-living communities, differences in host biology may lead to disparate patterns of community assembly and mechanisms of co-existence through time.

Here, we studied microparasite communities (*Theileria* spp.) throughout the lifetime of African buffalo to better understand the development of microparasite communities in a natural system. *Theileria* are tick-borne intracellular protists within the phylum Apicomplexa (Norval et al., 1992). African buffalo are reservoir hosts for three species clades (*T. taurotragi, T. mutans, T. velifera*), which also infect cattle at the wildlife-livestock interface and cause economically significant morbidity and mortality (Mans et al., 2016). In the buffalo host, all *Theileria* have a life stage in which they infect lymphocytes (a type of white blood cell) and red blood cells (Norval et al., 1992). However, *Theileria* within the *T. taurotragi* species clade (*T. parva, T.* sp. (bougasvlei), *T.* sp. (buffalo)) primarily replicate within white blood cells of the mammalian host before transfer to red blood cells for uptake by the tick vector; whereas *Theileria* within the *T. mutans* and *T. velifera* clades primarily replicate within red blood cells of the mammalian host (Norval et al., 1992). Protective immunity against *T. parva* (in the T. taurotragi clade) is largely mediated by CD8+ cytotoxic T cells and CD4+ cells associated with Type 1 T helper cells (Baldwin et al., 1992; McKeever et al., 1994) – both of which are typical immune response to intracellular parasites (Janeway et al., 2001). Overall, while initial infection by *Theileria* initiates a broad, non-specific, intracellular immune response, adaptive immunity against *Theileria* is complex and taxon-specific (MacHugh et al., 2009; Morrison, 1996).

Individual African buffalo are commonly infected concurrently by up to 12 closely related subtypes of *Theileria*. parasites and infection has been detected in animals at all life-stages (e.g., calves, juveniles, adults) (Combrink et al., 2020; Glidden et al., 2020; Henrichs et al., 2016). Many of these parasite subtypes persist through the host's adulthood, co-existing long-term despite close phylogenetic relatedness of parasite subtypes (Glidden et al., 2020). This raises the question how *Theileria* spp. avoid the effects of competition for shared resources (blood cells), and apparent competition due to cross-immunity among subtypes (blood cells) (Budischak et al., 2018; Raberg et al., 2006); and more generally, how community succession operates in this parasite community.

We longitudinally sampled a herd of African buffalo, containing newborn calves to adult animals, to ask: (1) do microparasites display life-history variation (i.e., variation in strategies used by the microparasites to replicate and persist within individual buffalo and the population) and patterns of succession congruent with classical succession theory?; (2) is succession in these parasite communities driven by the inter-specific facilitative or competitive mechanisms observed in free-living systems? We hypothesize that *Theileria* follow predictable patterns of colonization (e.g., age at first infection) as microparasites interact with each other and the host immune response to infect and replicate within naïve hosts. We expect that competition and facilitation is strongest in juvenile hosts who have lower blood cell counts and lack adaptive immune responses to the full suite of *Theileria* subtypes, and that the dynamics of the interaction network will help to illuminate mechanisms underpinning patterns of *Theileria* assembly and co-existence.

Methods

Data collection

Study system and sample collection

African buffalo included in this study were located in a 900-hectare enclosure within the Kruger National Park (KNP) a 19,000 km^2 preserve, located in northeastern South Africa (S 24 23' 52", E 31 46' 40"). The enclosure is entirely within the KNP and has numerous other wild animals typical of the ecosystem (e.g., giraffe, zebra, warthogs, small mammals and small predators). However, the enclosure excludes mega-herbivores (rhino, hippo, elephant) and large predators (lion, leopard). Study animals graze and breed naturally and find water in seasonal pans and man-made (permanent) water troughs. In extremely dry conditions, supplemental grass and alfalfa hay was supplied. A total of 66 African buffalo were included in the study. However, the size of the study herd fluctuated through time due to natural births and deaths. At any given time, the herd of African buffalo consisted of around 50 buffalo.

The herd was sampled at two-three month intervals from February 2014 – December 2015 (capture time points = 10). Animal capture and sedation protocols have previously been described by (Couch et al., 2017). The study was conducted under South Africa Department of Agriculture, Forestry and Fisheries Section 20 permits Ref 12/11/1, ACUP project number 4478 and 4861,

Onderstepoort Veterinary Research Animal Ethics Committee project number 100261-Y5, and the Kruger National Park Animal Care and Use Committee project number JOLAE1157-12.

For animals born during the study, their birth date was recorded, and their age was tracked through the study. For animals born before the start of the study, the precise age of animals estimated to be less than 2 years old was assessed from body length and horn length, the precise age of animals estimated to be between 2-5 years old was assessed based on incisor emergence patterns, and the precise age of animals estimated to be greater than 5 years old was assessed from tooth wear of incisor one (Jolles, 2007). Animals ranged from zero months to 19.50 years (234 months) old. Body condition was determined by assigning a score from 1 to 5 based on manually palpating four sites (ribs, hips, spine, and tail base); average score was used in all analyses (Ezenwa et al., 2009).

During each capture, 2 ml of whole blood was collected via jugular venipuncture directly into EDTA-coated vacutainers and stored on ice during transport. One milliliter of whole blood was pipetted into sterile microcentrifuge tubes and stored at −80°C until it was used for DNA extractions while the rest of blood was immediately used to measure red and white blood cell counts using an automated hematology analyzer (Vet ABC, Scil Animal Care Company).

Quantifying Theileria *spp. community abundance and subtype relative abundance*

Identically to Glidden et al. 2020, we used high throughput amplicon sequencing to detect and quantify read counts of *Theileria* subtypes using an 18S rRNA sequence specific to piroplasms

(*Theileria* spp. and *Babesia* spp.) (Gubbels et al., 1999). Additionally, we used a genus-specific qPCR to measure the total abundance of all *Theileria* subtypes (i.e., community abundance).

DNA was extracted from 200 µl of EDTA blood using DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. DNA extractions were shipped to the University of Melbourne, Australia, and stored at −20°C until further testing. The V4 hypervariable fragment (~500 bp) of the 18S rRNA gene of *Theileria* was targeted for amplicon sequencing. Briefly, PCR amplicons were generated using the RLBF (5′–GAG GTA GTG ACA AGA AAT AAC AAT–A3′) and RLBR (5′–TCT TCG ATC CCC TAA CTT TC–3′) primers (Gubbels et al., 1999) using the AmpliTaq Gold 360 mastermix (Life Technologies) in a thermal cycler (Veriti-384™; Applied Biosystem). The first PCR was run for the initial denaturation for 2 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 57°C, and 1 min at 72°C and a final extension of 8 min at 72°C. PCR amplicons were purified using magnetic beads and visualized on 2% E-Gel Agarose Gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher). The second PCR was performed to index the amplicons using the TaKaRa Taq DNA Polymerase (Clontech), and it was run for 2 min at 94°C, 15 cycles of 30 s at 94°C, 30 s at 57°C, 1 min at 72°C, and a final extension of 1 min at 72°C. The PCR products were then purified using magnetic beads, quantified by fluorometry (QuantiFlour® dsDNA System), and normalized. The equimolar pool of amplicons was cleaned again using magnetic beads to concentrate the pool and then measured using an Agilent High-Sensitivity D1000 Tape System (Agilent Technologies). The pool was diluted to 5 nM, and the molarity was confirmed again using the Tape System and sequenced on an Illumina MiSeq Reagent Kit v3 (600 cycle) using 2 × 300 base pairs paired-end reads. Positive (*Theileria orientalis*) and negative (no DNA template) controls were also included during each step of the experiment.

Sequence data was cleaned, filtered, and clustered using SeekDeep (V 2.5.1), a software that can obtain single base pair resolution from amplicon data (Hathaway et al., 2018). FASTQ files from all samples were processed using a within-sample relative abundance cutoff of 1% and the Illumina MiSeq tag, allowing no mismatches. Within the SeekDeep pipeline, sequences that were marked as likely chimeric were removed. Additionally, we removed any sequences that occurred once within the study as this would imply a unique sequence that occurred in one animal at one time point. Phred quality score of each consensus sequence was assessed in FastQC (V. 0.11.7). As the final PCR amplicon is ~460 bp, sequences were retained in the analysis if bases had an average Phred quality score >30 (1 error per 1,000 bases).

Bayesian inference (BI) and neighbor joining (NJ) analyses were conducted to identify sequences to *Theileria* subtype. First, a nonredundant database of all *Theileria* and *Babesia* subtypes known to infect African buffalo, as well as closely related species, was curated using the existing literature (Mans et al., 2015) and the NCBI database (GenBank). SeekDeep sequences and reference sequences were imported into Mesquite (V 3.51; Maddison & Maddison, 2008) and aligned using MUSCLE (V 3.8.31; Edgar, 2004). For the BI analysis, the likelihood parameters were based on the Akaike Information Criterion (AIC) test in jModeltest V.2.1.10 (Darriba et al., 2012). The likelihood parameters used were TrN + I + G (Nst = 6; rates = invariable + gamma). A Bayesian tree was constructed using the Monte Carlo Markov Chain analysis in MrBayes (V.3.1.2). Four simultaneous tree-building chains were used to calculate posterior probabilities for 2,000,000 generations, saving every 100th tree. A consensus tree was constructed based upon the final 75% of trees produced (burnin = 0.25%).

The NJ analyses were conducted in MEGA 7.0 (Kumar et al., 2016), and the nodes were tested for robustness with 10,000 bootstrap replicates. The data format was set to DNA, and gaps were treated as missing data. For the substitution model, substitution type was nucleotide, the method used was the number of differences, substitutions included were transitions and transversions, and rates among sites were uniform. The tree topology was checked for concordance. *Theileria* clades were considered supported if NJ bootstrapping values were >75% and Bayesian posterior probability values were >0.95. Subtype clades were considered supported if NJ bootstrapping values were >75%.

We additionally used a quantitative PCR analysis, described in detail in Glidden et al. 2020, to measure the % of blood cells infected by the *Theileria* genus as a whole (i.e., community abundance). We calculated community abundance from the qPCR result by following (Pienaar et al., 2011). Volume of whole blood used in the qPCR assay was calculated by multiplying the proportion of DNA extraction used in the assay by volume of whole blood used in the DNA extraction. Subsequently, the number of red blood cells in each reaction was then calculated by multiplying the volume (number of microliters) of whole blood used in each reaction by the number of red blood cells per microliter. As the 18S gene of *Theileria* is believed to have two copies per genome (Hayashida et al., 2012), copy number per sample (calculated from mean of duplicates) was divided by two to obtain the number of parasites per reaction. Parasites in each qPCR reaction per sample was divided by red blood cells per reaction and multiplied by 100 to obtain % parasitemia (i.e. community abundance).

Analytical methods

Characterizing patterns of succession and identifying signatures of trade-offs between dispersal and relative abundance in the climax community

To describe aggregate measures of community composition we used relative abundance of high-throughput sequencing read counts to quantify alpha diversity (Shannon Index, Inverse Simpson Index) and beta diversity (distance, in Euclidian space, from the community centroid). *Theileria* diversity within-hosts (alpha diversity) and among hosts (beta-diversity) were calculated using the *vegan* package (Oksanen, 2020) in R (R v. 4.0.2). As stated above, community abundance was measured as the number of blood cells infected with *Theileria* As the relationship between buffalo age (months) and our aggregate community metrics appeared non-linear, we used general additive mixed models to evaluate the relationship between age (independent variable, smooth variable) and the three measures of community composition (dependent variables, gaussian distribution). In each model, the smoothing basis was a penalized spline (p-spline). Animal ID and capture number were included as random intercepts. Model assumptions were tested using quantile-quantile plots and the distribution of deviance residuals. We tested for temporal autocorrelation by visually inspecting autocorrelation function and partial autocorrelation function plots. Additionally, we fit each model with an AR(1) and AR(2) error structure and used a log-likelihood test to evaluate if including a serial correlation structure improved model fit. We found that including a serial autocorrelation structure (AR(1)) improved model fit for the beta diversity (likelihood ratio comparing the null and AR(1) model = 6.70, p-value = 0.01) and parasite abundance model (likelihood ratio comparing the null and AR(1) model = 64.63, p-value < 0.01). Thus, we used

models with the AR(1) error structure for our final beta diversity and parasite abundance models. Additionally, community abundance was left skewed, thus we log-transformed percent cells infected for the analysis. Generalized additive mixed model analysis was conducted in *mcgv* (Wood, 2017) and model diagnostic plots were generated in *gratia* (Simpson, 2022).

We identified unique parasite life histories by analyzing change in mean relative abundance and prevalence of each *Theileria* species by buffalo age (months). The relationship between relative abundance of each *Theileria* subtype and animal age was non-linear, as such we fit this relationship using a penalized B-spline regression (Duan & Jiang, 2022). In the penalized B-spline regression, we first used generalized estimating equations (GEE) to model the compositionally and longitudinally dependent high-throughput sequencing read counts that represent the abundance of *Theileria* subtypes. To further estimate and distinguish the longitudinal profiles of the parasite abundances, we reparametrized the GEE by writing its original parameters as smooth functions of the animal age, which are then approximated by a linear combination of B-spline basis functions with unknown parameters. Finally, we computed the estimated values of these unknown parameters by applying penalized B-splines, i.e., maximizing the sum of the quasi-likelihood function from GEE and a penalty function on the B-spline coefficients that controls the smoothness of the B-spline functions (Duan & Jiang, 2022).

After we obtained the B-spline coefficients and thus the fitted longitudinal curves of the parasite relative abundances, we determined a few point estimates indicative of different life history strategies for each *Theileria* subtype: the age of first infection (defined as the age at which the relative abundance exceeds 0.01 for the first time), the maximum relative abundance, the age at

the maximum relative abundance, the relative abundance that becomes stable, and the age when the relative abundance becomes stable (defined as the age followed by the longest time interval whose slope of the fitted curve is less than 0.003). We also applied bootstrap to estimate the 95% confidence intervals for these point estimates, in which we took 1,000 random samples with replacement as the bootstrap samples.

Age-prevalence curves were created by binning animals within 6-month age categories and calculating the proportion of animals infected out of the total number of animals sampled for each subtype for each age class.

Standard errors for prevalence of each subtype for each age class were calculated by Eq. 1.:

$$SE = \frac{\sqrt{No.animals\ infected \times (1 - No.animlas\ infected)}}{No.animals\ sampled} \qquad \text{Eq. 1}$$

To identify signatures of trade-offs between life-history traits, we calculated pearson's correlation coefficients between life-history traits of each subtype (excluding rare subtypes). Specifically, we examined the correlation between age at first infection versus relative abundance at equilibrium, mean age at equilibrium versus relative abundance at equilibrium, and mean age at first infection versus mean age at equilibrium.

Describing the development of the Theileria spp. *interaction network through the lifetime of the animal*

Prior to analyzing *Theileria* interactions, we center-log transformed read counts (Gloor et al., 2017). Following (Karakoç et al., 2020), prior to analysis, missing data points (2% of data in the adult animal dataset) were linearly interpolated using the 'na.approx' function in *zoo* (Zeilis & Grothendieck, 2019).

We tested for non-linear, deterministic causal interactions between pairs of *Theileria* subtypes using convergent cross mapping (CCM), which detects information transfer from one variable to another using nonparametric state space reconstruction (Clark et al., 2015; Sugihara & May, 1990; Takens, 1981). If information transfer is detected, then we assume a causal association between subtypes (facilitation or competition), with the causal direction indicated by direction of information flow.

Since stable estimates from CCM typically require a time series of at least 30 sequential observations, we pooled time series across captured animals to fill in the state space manifold (Clark et al., 2015; Hsieh et al., 2008). We observed that subtype trajectories in young animals were quite different than in adult animals; as such we described differences in significant interactions detected during stages of succession, by running separate analyses for juvenile animals (< 3-years old) and adult and juvenile animals (> 3-years old). We selected the 3-year age cutoff as this is the buffalo age, on average, that all subtypes reached their average rank in climax communities (Table 1). Importantly for pooling replicates using CCM, the trajectories of subtypes were quite similar, but not identical, for animals within these age classes (Figures S2-3).

Embedding dimensions (E, time-lags used for state space reconstruction) were chosen using simplex projection which tests the ability of variables to predict their own dynamics through leave-one-out cross-validation. We chose E as the smallest dimension that is within 1% of the best predictive value across the dimensions tested (Clark et al., 2015; Sugihara et al., 2012; Sugihara & May, 1990; Ye et al., 2015). For each age category, our time series for each buffalo was ≤10-time steps; additionally, the shortest time series for the juveniles was 4 whereas the shortest time series for adult animals was 5. Thus, as the maximum embedding dimension should be the square root of the maximum time series length (Cheng & Tong, 1992) but one less than the shortest time series length (Clark et al., 2015), we choose a maximum E of 3 for juvenile animals and 4 for adult animals. We used mean absolute error (MAE) to test predictive ability. We included 29 animals in our adult animal analysis and 27 animals in our juvenile animal analysis. For the juvenile animals, we used all subtypes with the exception of rare subtypes *T. mutans*-undefined, *T.velifera*-undefined, and *T.* sp. (buffalo). For the adult animals, we used all subtype except for rare subtypes and *T. mutans* and *T. mutans* MSD as these were found in less than 1/3 of adult buffalo samples (Figure 2).

To detect interactions with time lagged effects, we applied CCM with varying prediction lags (-1 to 0). Significance of results was determined using nonparametric bootstrapping by sampling from the observations with replacement and recalculating statistics for 10,000 iterations to determine 95% confidence intervals. In cases where CCM identified significant causal interactions, we selected the best time lag as the time lag with the best predictive ability (quantified using MAE) at the largest library length. Next, for significant interactions, we computed a series of Jacobian-like matrices to determine the direction and strength of species interactions in each time-step using

S-mapping, which is a locally weighted multivariate linear regression (Deyle et al., 2016). S-mapping predicts dynamics from the reconstructed state based on E interacting predictor variables, which can be either time-lagged observations of the target subtype (intraspecific effects), or observations of other subtypes. Variables for S-mapping were selected based on the number of E of each target variable. If the number of E was larger than the number of variables causing the target variable, predictor variables were complemented with the time-lagged observations of the target variables. The linear approximation is conducted locally in state-space, giving greater weight to the target points near the current state, and can be interpreted as localized linear approximations of the community matrix at different times. Nonlinearity parameter (θ) for S-mapping was defined based on univariate predictive ability (Sugihara & May, 1990).

We evaluated model assumptions (interactions are non-linear and deterministic) by plotting Pearson's correlation coefficient and MAE by library size, deeming an interaction acceptable to use for the analysis if predictive ability increased and saturated with library size (Clark et al., 2015). Typically, in empirical dynamical modeling, model performance is evaluated by using the first half of the time series to reconstruct the state space and the second half of the time series to evaluate model prediction ability. For our study, we used relatively short time segments (maximum time steps = 10) with a relatively small number of replicates (~30) so reserved the entirety of the time segments to reconstruct the state space. Thus, to evaluate model performance, we randomly subsampled animals at increasing sample sizes (by 2 animals per subsample) and calculated a series of summary statistics to evaluate model performance. For the adult and juvenile analysis, we observed that the average root-mean-square-error of significant interactions decreased and to a stable plateau with sample size (Text S1, Figures S4-5); thus, we consider our results robust and

useable. We performed empirical dynamical modeling using the R package *rEDM* [66] and *EDMhelper* (Karakoç & Clark, 2022) in R (version 3.6.1). We visualized our networks using *igraph* (Csardi & Nepusz, 2006).

Results

We sampled a herd of 66 African buffalo, ranging from newborn animals (0 months) to 19-year-old animals, every 2-3 months for two years. Upon their inclusion in the study, 18 animals were less than 6 months old, 23 animals were subadults between 1 year old and 4 years old, and 24 animals were greater than 4.5 years old (reproductively mature adults). In the analysis, we included 488 samples (one per buffalo per capture), with each buffalo sampled and average of 7.5 (95% confidence interval: 7.19 – 7.73) times. On average, at a single time point, buffalo were infected with 7.3 *Theileria* subtypes (95% confidence interval: 7.18 – 7.40). However, as described in detail below, the number of *Theileria* subtypes infecting a buffalo at a given time varied substantially with host age.

Theileria *communities follow predictable patterns of succession, characterized by life history variation among subtypes and a colonization – persistence trade-off.*

We found clear, highly predictable successional patterns in *Theileria* community structure: host age had a significant, non-linear effect on alpha diversity (effective degrees of freedom (edf) = 8.69, p-value < 0.01; Figure 1a; Figure S1), beta diversity (edf = 8.70, p-value < 0.01, Figure 1b), and community abundance (edf = 3.70, p-value < 0.01; Figure 1c). *Theileria* diversity in individual

hosts (i.e., alpha diversity) increased and saturated with buffalo age, reflecting sequential colonization of young buffalo by the various *Theileria* subtypes. However, *Theileria* communities among buffalo resembled each other more and more closely with increasing host age, followed by a slow increase in the oldest animals; that is, beta diversity mostly declined with host age, suggesting succession towards a predictable climax community of *Theileria*s in African buffalo. The fraction of host blood cells infected by *Theileria* parasites -- *Theileria* community abundance -- peaked during early succession and declined towards a stable equilibrium of moderate infection levels in adult buffalo.

We similarly found clear and predictable patterns in individual subtype occupancy and abundance, with some evidence of life-history trade-offs within the *T. mutans* clade. Individual buffalo typically acquired most subtypes of *Theileria* by the time they were one year old (Figure 2, Table 1). After the host was > 3 years old, relative abundance of most subtypes reached equilibrium-like, steady relative abundances (Figure 2, Table 1). However, we found substantial variation in subtype life histories, driven by variable subtype dynamics in young buffalo and differential abundances in climax assemblages (Figure 2).

T. velifera, T.velifera B, *T. mutans,* and *T. mutans* MSD appear to be early colonizers as they were present in most calves at their first sampling time point. Early infection with these subtypes was quite ubiquitous with >75% of animals 0-18 months old infected with all four subtypes (Figure 2a). However, they differed in their persistence patterns: *T. velifera* and *T.velifera* B infected calves at high relative abundances and then persisted at moderate relative abundances in juvenile and adult animals (Figure 2b). Prevalence across all age groups was 100% for *T. velifera* and >75% for *T. velifera* B (Figure 2a). In contrast, *T. mutans,* and *T. mutans* MSD were commonly cleared

from the host after initial infection: About half of animals cleared *T. mutans* by the time they were 24-30 months old, and the majority of animals cleared *T. mutans* MSD by the time they reached 18-24 months of age (Figure 2). As such, these early-arriving subtypes can be divided into two life history groups: Early-persistent (*T. velifera, T.velifera* B) and early-ephemeral (*T. mutans, T.mutans* MSD).

T. mutans-like 1, *T. mutans*-like 2, and *T. mutans*-like 3 appear to be late colonizers, with average age of first infection > 5 months, and *T.mutans*-like 3 not infecting animals until they were on average 10 months old (Table 1). Starting at 18-24 months of age, 100% (standard error = 0) of animals were infected with these three subtypes, suggesting that animals harbored persistent infections throughout their lifetime (Figure 2a). *T. mutans*-like 3 and *T. mutans*-like 1 had the highest average relative abundance in *Theileria* climax communities indicating that these two subtypes are commonly the most abundant within adult animals (Table 1; Figure 2b).

T. sp. (bougasvlei) and *T. parva* exhibit more variable patterns. The average age of first infection was > 5 months, however, the 95% confidence interval around this estimate is quite large (Table 1; Figure 2b). Likewise, the mean relative abundance of *T.* sp. (bougasvlei) in climax assemblages was very variable (95% CI = 0.02-0.35), and variable prevalence for these subtypes indicates that some hosts may gain and lose infections throughout their lifetime (Figure 2). Altogether, late colonizing subtypes can also be divided into two life history groups: late-persistent (*T. mutans*-like 1, *T.mutans*-like 2, *T.mutans*-like-3: *T. mutans*-like 1-3) and late-ephemeral (*T. parv*a, *T.* sp. (bougasvlei)).

Lastly, *T. velifera* undefined, *T. mutans* undefined and *T.* sp. (buffalo) only infected a small fraction of the host population and did not exhibit clear within-host infection patterns (Figure 2). These subtypes appear to infect buffalo sporadically, perhaps spilling over from other host species that may be more central to their dynamics. As such, these subtypes exhibit no clear life history patterns in buffalo.

When comparing traits, we found a strong positive correlation between mean age at first infection and mean relative abundance at equilibrium (Figure 3A, $\rho = 0.52$); as well as mean age at equilibrium and relative abundance at equilibrium (Figure 3B, $\rho = 0.63$). Thus, *Theileria* subtypes that infect hosts early tend not to be as abundant in climax *Theileria* communities as later colonizing subtypes, suggesting a trade-off between dispersal or colonization ability and competitive dominance in this clade of microparasites. This pattern is primarily driven by subtypes in the *T. mutans* clade, as *T. mutans* and *T. mutans* MSD infected all young animals early but were absent in the majority of adult animals (>70%). We found almost no correlation among mean age at first infection and mean age at equilibrium (Figure 3C, $\rho = 0.02$).

Theileria spp. *interaction networks are dynamic and non-linear, with young animals infected by more complex networks than adult animals*

We used empirical dynamical modeling to quantify interaction networks in in juvenile (<= 3-year-old animal) and adult animals (>= 4-year-old animals) (Figure 4; Table 2; Table S1). We found that interaction networks are non-linear and dynamical, decreasing in complexity with age.

Theileria interaction networks differed dramatically between transient successional communities observed in young buffalo, and climax *Theileria* communities found in adult hosts. Interactions between *Theileria* subtypes were far more common in juvenile hosts. The *Theileria* interaction network in juvenile buffalo was composed of 23 detectable interactions, including both negative and positive interactions that spanned taxonomic and life history groups. The strongest interactions were generally positive and occurred between *Theileria* subtypes sharing similar life history strategies: early arriving (both persistent and ephemeral) and late-persistent subtypes. A web of negative interactions connected *Theileria* subtypes with contrasting life history patterns and taxonomic affiliations (Table 2; Figure 4). On average, the late colonizing subtypes, *T. mutans*-like 1 and *T. mutans*-like 3, had a strong negative effect on the early ephemeral subtype *T. mutans* MSD, while early persistent subtypes in the *T. velifera* clade had a strong negative effect on early ephemeral subtype *T. mutans.*

By contrast, the detectable parasite interaction network in adult animals was composed of only three positive interactions within the *T. mutans* species clade and late-persistent life history group (Table 2; Figure 4). The juvenile and adult network overlapped only minimally, sharing a single interaction (*T. mutans*-like 1 had a consistent positive effect on *T. mutans*-like 3). In summary, *Theileria* communities in juvenile animals vary in richness and are made up of diverse interaction networks, whereas *Theileria* communities in adult buffalo have ubiquitously high richness with a low density of detectable interactions.

Discussion

Our work provides a picture of the development of microparasite community dynamics over the lifetime of a wild, long-lived mammalian host. In general, we found that micro-parasite succession patterns reflect those of many free-living communities and follow predictable patterns of succession. Specifically, we found that, within-host parasite diversity and among-host community predictability increased with host age: alpha diversity increased while beta-diversity decreased. Underpinning these results, we found that colonization and persistence patterns of individual parasite subtypes were both consistent across individual buffalo and distinct, representing unique life-history strategies and deterministic population dynamics. Among life-history groups, we observed negative correlation between timing of colonization and persistence, reflecting a competition-persistence trade-off. However, unlike free-living communities, we found that the number of detectable interactions decreased with host age and only positive interactions were present in adult communities. We found that the infection intensity (% of blood cells infected by *Theileria*) decreased with host age, never exceeding 0.5% of cells infected in animals less than three years old, suggesting that *Theileria* may not be resource limited and thus the change in interaction network and colonization-persistence dynamics may be regulated by host immunity.

In many free-living communities, within-host species richness and evenness (alpha diversity) non-linearly increases following the creation of a habitat patch (Miller & terHorst, 2012; Patiño et al., 2018; Quesada et al., 2009; Sattler et al., 2010). We found this exact pattern in our buffalo population: buffalo calves were infected with 2-3 *Theileria* subtypes, whereas animals greater than three years old were typically infected with 6-10 subtypes, with a small peak in alpha diversity (measured using both the Shannon index and Inverse Simpson) around the three-year mark. The

composition of *Theileria* communities became more similar among animals as hosts aged (i.e., declining beta diversity), indicating that the composition of *Theileria* communities in adult animals is largely predictable, similar to climax communities in free-living systems (Dini-Andreote et al., 2014; Guariguata & Ostertag, 2001; López-Martínez et al., 2013; Martins et al., 2018). We observed a colonization persistence trade-off where some subtypes consistently infected animals less than two months old but were cleared by the time animals were greater than three years old, while other subtypes only infected older animals but persisted at high relative abundances throughout the lifetime of the animal. This trade-off resembles colonization-competition trade-offs observed in numerous ecological systems (e.g., Cadotte et al., 2006; Rodríguez et al., 2007; Smith et al., 2018; Stanton et al., 2002) or may reflect the outcome of niche partitioning that is correlated with temporal changes in the habitat patch (Dini-Andreote et al., 2014). We found that, in sum, interactions between life-history groups displaying the largest colonization-persistence trade-offs (early ephemeral and late persistent subtypes) were negative, supporting a competition-colonization trade-off. Further, early persistent subtypes (*Theileria velifera* clade) negatively affected subtypes across multiple clades and life-history groups in young buffalo but were not affected by any subtypes themselves, displaying a uniquely aggressive life-history strategy. Finally, in contrast, late ephemeral groups were negatively influenced by both early ephemeral and early persistent groups in young animals and tended to colonize buffalo once relative abundance of these two groups decreased.

Classical succession theory is based upon the concept that habitat patches become increasingly crowded through time, placing restraints on resource availability and ultimately driving interspecific competition (Tilman, 1985). If *Theileria* communities were regulated by host

resources (blood cells), we would expect *Theileria* abundance, or % of host cells infected, to increase with host age as a more diverse parasite community composed of increasingly competitive players might exploit the available host resources more efficiently. However, we found that community abundance decreased with host age. The most parsimonious hypothesis is that decreased parasite abundance (% of cells infected) is due to the development of more effective acquired immunity and stronger ability to regulate parasite proliferation. The host's adaptive immune responses represent a process fundamentally distinct from the competitive and facilitative interactions that shape free-living communities (Rynkiewicz et al., 2015). Parasite communities' "habitat patches" (i.e., hosts) progressively develop specific resistance against them, effectively reducing parasite reproduction and survival over time. There is not a clear analogy in free-living communities, as top-down regulation by predators does not typically switch through time from distinctly generalized predation to highly specialized, targeted killing across a diverse suite of taxa. In the *Theileria* system, early successional communities may be regulated by broad, innate immunity of naïve hoses whereas late successional communities may be regulated by highly subtype specific adaptive immunity (Doolan et al., 2009; Simon et al., 2015). As such, many of the subtype life-histories observed may reflect their unique interactions with the host's immune system through time, and the strategy adapted to ensure transmission within the host population. For example, the colonization-persistence trade-off observed in the *T. mutans* clade may be the result of early colonizers invading and quickly replicating within hosts with naive immune systems, whereas persistent subtypes may play a slower strategy based on evasion of specific immune responses in adult hosts.

We found that the change in host interaction network with host age largely supports the idea that *Theileria* life histories and interactions primarily reflect their interactions with the host's immune responses rather than competition for shared resources. Interaction networks in young animals were dense and included positive and negative interactions, whereas interaction networks in adults only consisted of a few positive interactions within the *T. mutans* clade. This contrasts with free-living communities, where late-stage communities are often characterized by dense negative inter-taxon interactions due to intense competition for limiting resources (e.g., Coyte et al., 2015; Miller & terHorst, 2012) and / or apparent competition among taxa sharing generalist consumers. In the *Theileria* parasite community we studied, competitive interactions appear limited to early successional stages, while top-down regulation in climax communities may be mediated by specific immune responses to each subtype – in effect, specialist predators targeting each taxon in the community. Interestingly, increases in adaptive immunity in this parasite community should thus be functionally equivalent to an increase in the density of negative trophic interactions in a free-living system, a process which can promote co-existence of closely related species (Karakoç et al., 2020; Terborgh, 2015; Wallach et al., 2015). Future work quantifying immune effectors (e.g., specific antibodies) against each *Thelieria* subtype, and / or manipulating the strength of adaptive immune responses mounted by the host (e.g., by treating with steroids to suppress immune responses (Spaan et al., 2017), could help to test these ideas.

Our findings have several limitations. Species interactions are notoriously difficult to quantify in observational systems and, while our longitudinal sampling design allowed for advanced causal inference, we may have failed to detect weak interactions due to the length of our sampling interval (Fenton et al., 2014). Additionally, we may have missed some parasite interactions in adult animals

as communities were less variable and less suitable for manifold reconstruction than those observed in juvenile animals. While it would be ideal to use experimental approaches to test these ideas, this would be technically and logistically quite challenging in this study system. However, increasing temporal (the number of sampling time points) and spatial (the number of animals) resolution in similar longitudinal observational studies could help with detection of weak interactions, especially in adult hosts (Clark et al., 2015). These limitations notwithstanding, it appears clear that early successional communities in our host – microparasite study consist of much denser interaction networks than late-successional communities. Finally, we represent the network as two snapshots in time. Through empirical dynamical modeling, we calculated an interaction coefficient per buffalo per time step. Next steps include determining how host traits (e.g., immune mediators) influence changes in interaction strength over time.

Overall, individual *Theileria* subtypes follow predictable patterns of succession, leading to change in community diversity reminiscent of free-living systems. However, unique features of the mammalian immune response may drive disparities in the ecological-evolutionary mechanisms shaping parasite life-histories and co-existence.

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Data accessibility statement

Theileria relative abundance data and meta-data are stored on Dryad: https://doi.org/10.5061/dryad.j6q573nk1. Unique haplotype data are stored in NCBI GenBank GenBank Accession Numbers: MK792966–MK92994.

Author Contribution

CKG, AEJ, BB designed research. CKG, AEJ, BB, CD, YJ performed the research. CKG, CD, YJ, CK, AJ contributed new analytical tools. CKG and CD analyzed data. CKG, AEJ, CK, CD, and YJ wrote the paper. All authors provided feedback and approved the final version of the manuscript.

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Table 1. Point estimates for *Theileria* life history infection patterns. Age (months) 1st infection, age (months) at equilibrium, relative abundance at the equilibrium. Estimates include mean [95% confidence interval].

species clade	subtype	age 1st infection	age at equilibrium	relative abund at equilibrium
T. velifera	*T. velifera*	0.04[0.02-0.06]	24.37[9.92-38.81]	10.29[7.25-16.24]
	T. velifera B	0.04[0.02-0.06]	25.25[9.13-41.36]	0.04[0-1.25]
	T. velifera UD	NA	NA	NA
T. mutans	*T. mutans*-like 1	6.19[2.4-9.98]	21[3.35-38.65]	59.88[42.4-59.88]
	T. mutans-like 2	1.59[0-6.45]	15.79[0.35-31.22]	56.17[40-59.88]
	T. mutans-like 3	7.8[3.12-12.47]	22.68[7.92-37.44]	53.87[37-59.88]
	T. mutans MSD	0.04[0-0.09]	17.47[7.66-27.28]	6.84[4.79-10.85]
	T. mutans	0.04[0.02-0.06]	19.38[8.3-30.46]	0.36[0-3.25]
	T. mutans UD	NA	NA	NA
T. taurotragi	*T.* sp. (bougasvlei)	0.7[0-3.15]	25.1[7.19-43]	34.01[20.71-59.88]
	T. sp. (buffalo)	11.18[0-37.26]	0.04[0-6.84]	59.88[40.93-59.88]
	T. parva	1.99[0-5.12]	12.13[0.78-23.47]	19.61[9.5-39.42]

Table 2. *Theileria* interaction network summarized by life-history strategies. Numbers represent mean significant interaction coefficients. Row causes column.

Juvenile network

	EE	LP	EP	LE
Early ephemeral (EE)	1.01	-0.07	0.08	-0.61
Late persistent (LP)	-0.96	1.51	0	0.01
Early persistent (EP)	-0.02	-2.38	0.01	-0.34
Late ephemeral (LE)	0	0	0	0

Adult network

	EE	LP	EP	LE
Early ephemeral (EE)	0	0	0	0
Late persistent (LP)	0	1.36	0	0
Early persistent (EP)	0	0	0	0
Late ephemeral (LE)	0	0	0	0

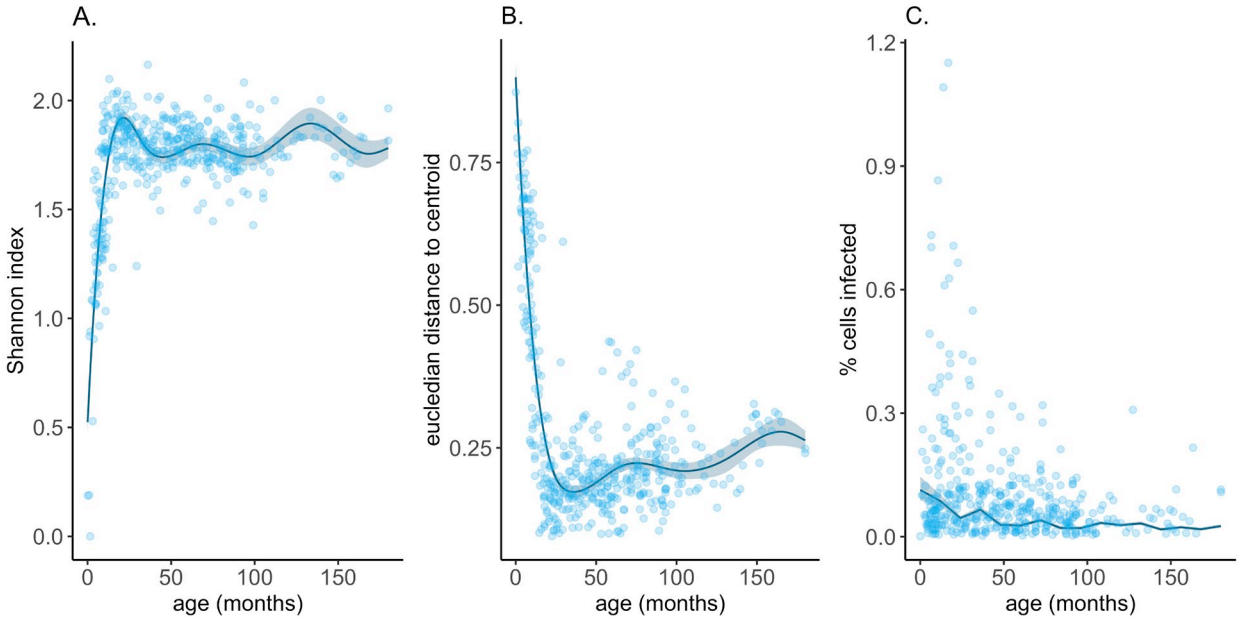

Figure 1. Change in aggregate community metrics with age. Points represent observations and lines represent generalized additive mixed model predictions (shaded regions represent standard error). **(A)** Alpha diversity calculated as Shannon diversity index; **(B)** Beta diversity calculated as the Euclidean distance to the centroid; **(C)** Community abundance calculated as percent of buffalo blood cells infected by all *Theileria* subtypes (% parasitemia).

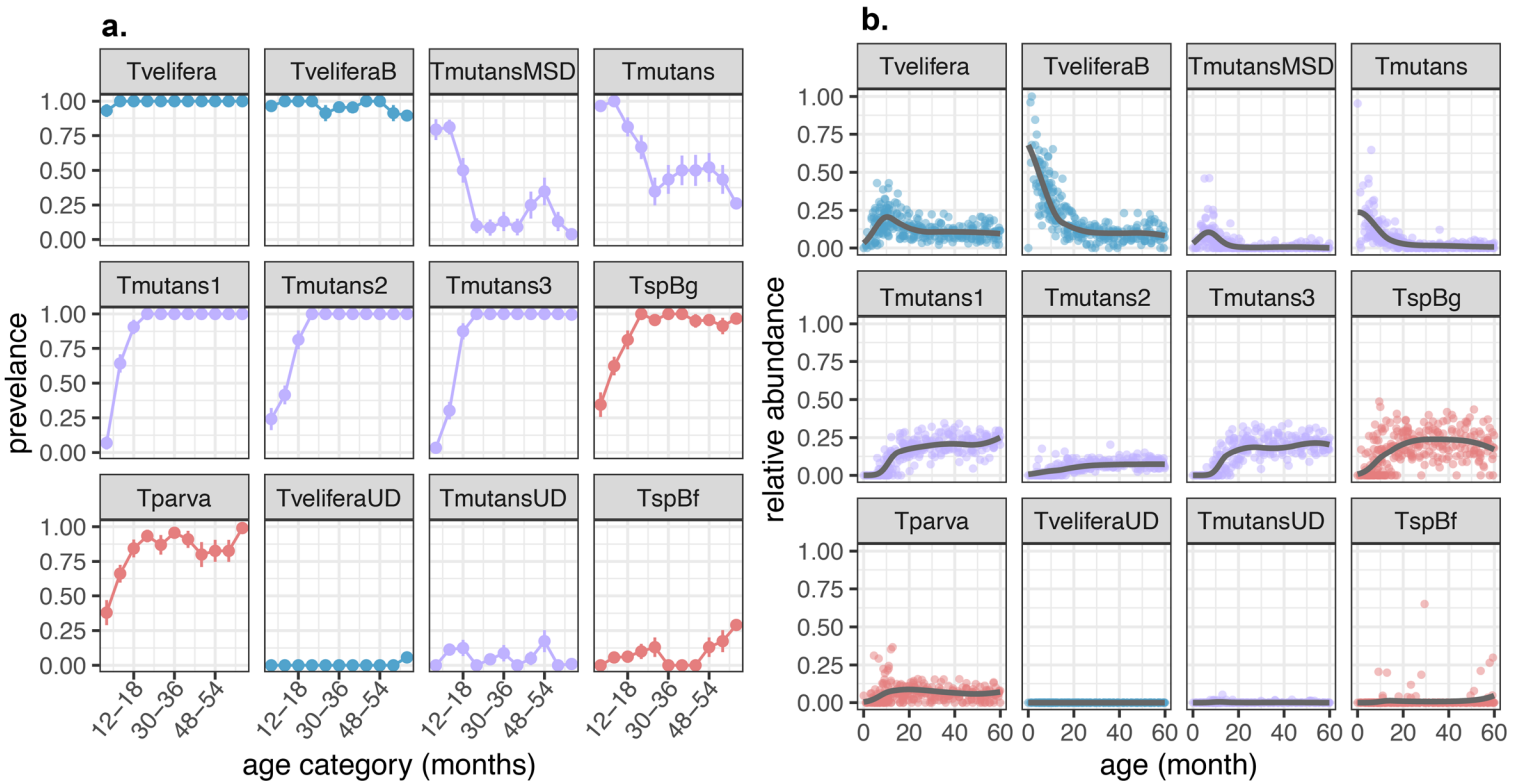

Figure 2. Life history variation in *Theileria*. **(A)** Population-level prevalence curves for each subtype: Prevalence of each subtype within each age bin. As our study was a repeated sample, longitudinal design, subtypes that are highly prevalent in early age bins but are absent or at low prevalence in later age bins, indicate that they were cleared from the host as the host age. **(B)** Within-host relative abundance by age: The points represent data whereas the lines represent estimates for average relative abundance by age fit with a basis-spline regression. Data were weighted by animal ID to account for the repeated measures study design. In **(A)** and **(B)**, red points include subtypes in the *T. taurotragi* species clade, blue points include subtypes in the *T. velifera* species clade, and purple points include subtypes in the *T. mutans* species clade.

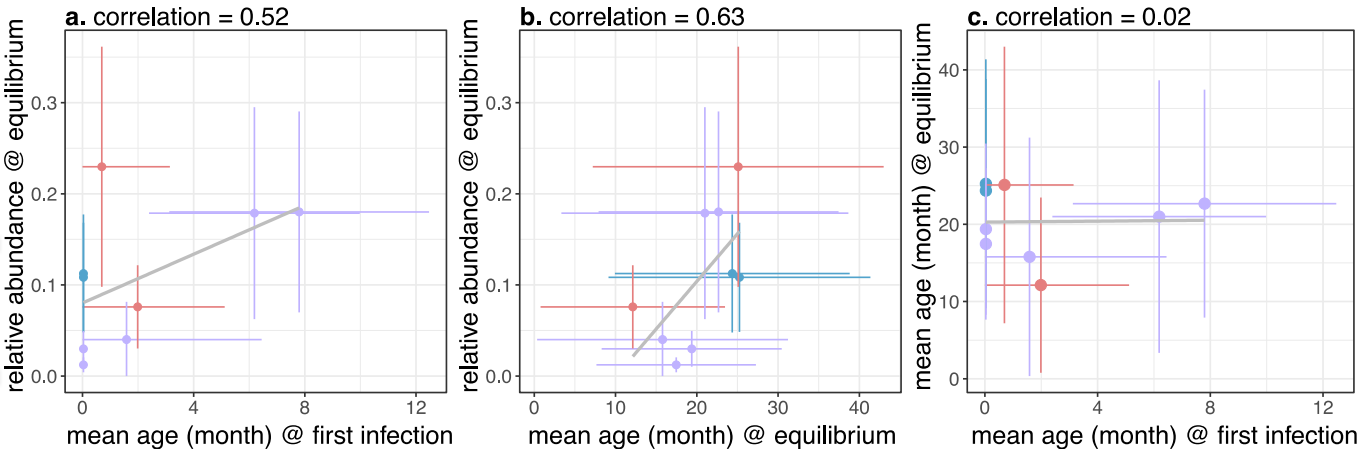

Figure 3. Correlations among average life-history variables. Blue points are subtypes in the *T. velifera* clade, purple points are subtypes in the *T. mutans* clade, and red points are subtypes in the *T. taurotragi* clade. Points represent means and bars represent 95% confidence intervals. Rare subtypes (*T. velifera* undefined, *T. mutans* undefined, and *T.* sp. (buffalo)) are not included. In all figures, red points include subtypes in the *T. taurotragi* species clade, blue points include subtypes in the *T. velifera* species clade, and purple points include subtypes in the *T. mutans* species clade.

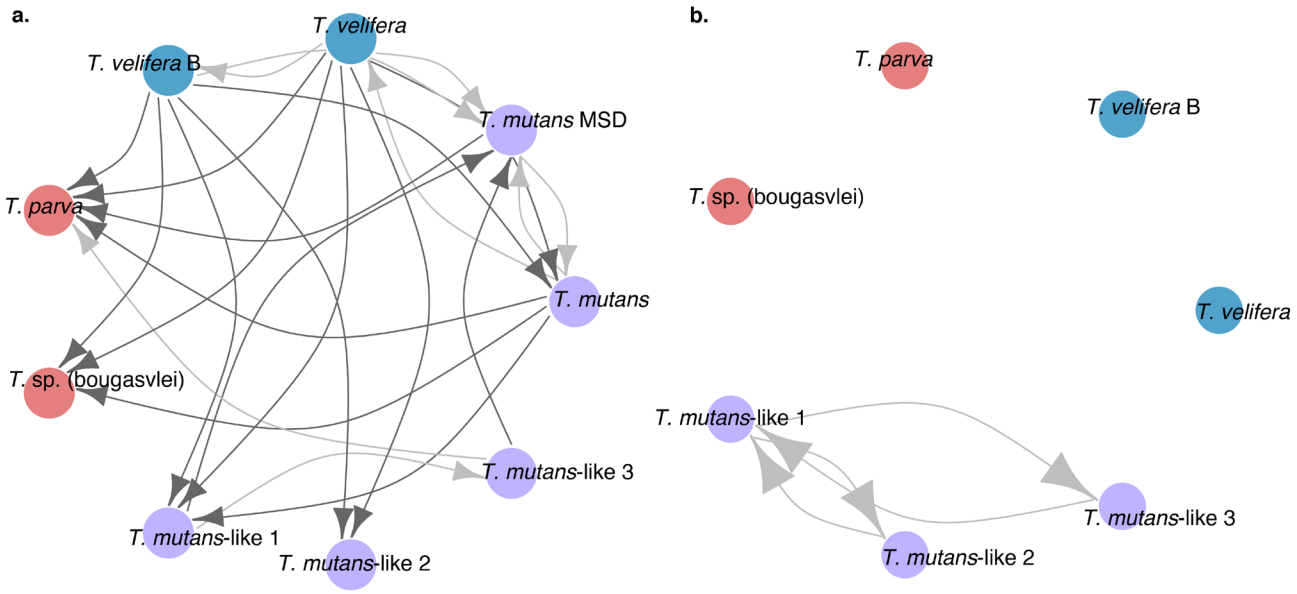

Figure 4. *Theileria* interaction network for (A) juvenile and (B) adult animals. Only significant interactions ($p < 0.05$) are depicted. Edges represent significant interactions. Arrows indicate the direction of the interaction ($subtype_A \rightarrow subtype_B = species_A$ causes $species_B$); positive interactions are light grey whereas negative interactions are dark grey. Nodes represent subtypes. Blue nodes are subtypes in the *T. velifera* clade, purple nodes are subtypes in the *T. mutans* clade, and red nodes are subtypes in the *T. taurotragi* clade. The *T. velifera* clade are early, persistent subtypes; *T. taurotragi* clade are late, ephermeral subtypes; *T. mutans* clade is broken up into early ephemeral (*T. mutans* , *T. mutans* MSD; not in b. due to low prevelance in adults) and late persistent (*T. mutans*-like 1-3).