Fitness costs of parasites explain multiple life history tradeoffs in a wild mammal

Gregory F Albery, Daniel H Nussey, Josephine M Pemberton

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK, EH9 3FL

*gfalbery@gmail.com

Abstract

A longstanding prediction of life history theory states that parasitism can provide a mechanistic link between current and future fitness, thereby mediating life history tradeoffs. Demonstrating this mechanism in the wild involves linking a reproductive cost for parasitism with a cost of parasitism for fitness, which requires high-resolution longitudinal monitoring of individuals. Here, we examined associations among reproductive investment, immunity, parasitism, and fitness-related traits in a wild population of individually identified red deer (*Cervus elaphus*). Using path analysis, we constructed and analysed possible links among these traits, particularly investigating whether costs of lactation for survival and fecundity were likely to act through increased parasitism. Lactation was associated with increased strongyle parasitism, which was then associated with decreased subsequent fitness for all four fitness-related traits we examined. In addition, lactation was directly associated with later parturition date the following year, and increased helminth-specific antibody levels were associated with increased probability of reproduction. This study offers observational evidence for parasite mediation of multiple life history tradeoffs in a wild mammal, and supports the value of considering life history investment, immunity, and parasitism simultaneously when examining fitness consequences in disease ecology.

Introduction

A fundamental tenet of life history theory states that reproduction should be costly for future fitness, yet the mechanisms behind such tradeoffs are poorly understood (Williams, 1966; Stearns, 1989). It has been suggested that reproductive costs may act through parasites, where increased reproductive investment diverts resources away from immunity, resulting in increased parasite burdens, which reduce subsequent fitness (Williams, 1966; Harshman and Zera, 2007). Parasite mediation of life history tradeoffs involves two necessary components: that reproduction increases parasitism, and that parasites cause harm or require resources to combat them, reducing subsequent fitness. There is abundant evidence for each component of this theory across a range of taxa: firstly, life history investment is often associated with weaker immune investment (Neggazi *et al.*, 2016; Rödel *et al.*, 2016; Krams *et al.*, 2017) or with increased parasitism (Festa-Bianchet, 1989; Cizauskas *et al.*, 2015; Debeffe *et al.*, 2016). Secondly, parasitism often decreases probability of survival (Coltman *et al.*, 1999) or reproduction (Albon *et al.*, 2002; Vandegrift *et al.*, 2008; Hughes *et al.*, 2009). Despite evidence for both in isolation, reproduction-associated increases in parasitism have rarely been linked to downstream fitness consequences in the same study to provide evidence for a parasite-mediated life history tradeoff.

Parasites can be costly for fitness by directly harming the host and sapping its resources - however, immunity is also costly, in that immune responses use resources and cause immunopathology which can likewise reduce fitness (Ilmonen *et al.*, 2000; Raberg, 2000). The cost of parasites must therefore be weighed against the cost of immunity itself: it is difficult to predict *a priori* whether increased fitness should result from higher, lower, or intermediate immune responses, yet it is still relatively rare that both immunity and parasitism are quantified in studies of fitness consequences (Viney *et al.*, 2005; Graham *et al.*, 2011). Furthermore, while it is true that reproduction, immunity, and parasites all compete for host resources, mechanisms governing life history tradeoffs are time-staggered rather than occurring simultaneously. First, reproduction reduces allocation to immunity (Sheldon and Verhulst, 1996). This weaker immunity, plus potentially increased exposure associated with altered behaviour in reproductive individuals, results in higher parasitism (Knowles *et al.*, 2009; Albery *et al.*, 2018b). Finally, future fitness is reduced by damage from parasites (Harshman and Zera, 2007; Graham *et al.*, 2011). This combination of mechanism comprises an “indirect” cost of reproduction acting through parasites. Additional (direct) costs of reproduction can simultaneously act through other mechanisms such as reduced condition, hormonal or phenological regulation, or damage caused by oxidative stress (Stjernman *et al.*, 2004; Harshman and Zera, 2007; Speakman, 2008). Thus, all three of reproductive investment, immunity, and parasitism can have independent effects on fitness.

In order to consider parasites' place in tradeoffs between life history components, a distinction must be made between "input" costs of life history investment for immunity/parasitism, and "output" costs of immunity/parasitism for fitness. This distinction is rarely made explicitly, so direct reproductive costs have rarely been compared directly with the costs of immunity or parasitism in mediating life history tradeoffs.

General life history tradeoffs can be hard to demonstrate observationally due to the confounding effects of individual quality or resource acquisition rate (van Noordwijk and de Jong, 1986). Specifically concerning immunity, it is possible that individuals in poor condition both exhibit weaker immunity and do not reproduce, resulting in a reversed pattern at the population level in which reproductive individuals are actually less likely to die from parasites (e.g. Lynsdale *et al.,* 2017). Similarly, causality may be reversed: for instance, pregnancy can negatively affect immunity (Weinberg, 1984), but more highly parasitized individuals may be less likely to become pregnant in the first place (e.g. Hughes *et al.* 2009). Experimental approaches are therefore preferred when examining life history tradeoffs and their immune correlates, and clutch size manipulations have commonly been employed in birds to investigate these questions (Knowles *et al.*, 2009). However, reproductive effort is hard to manipulate in wild mammals (Rödel *et al.*, 2016), and in any case we are not aware of any published experiment in either birds or mammals in which both reproductive effort and parasite burdens were manipulated in wild animals to investigate causal links.

The wild red deer (*Cervus elaphus*) on the Isle of Rum exhibit a life history tradeoff wherein lactating to a calf until its first winter reduces a female's future reproduction and survival probability (Clutton-Brock *et al.*, 1989; Froy *et al.*, 2016). This reproductive cost has long-lasting fitness consequences (Moyes *et al.*, 2006). Females that give birth to a calf that dies within the first few months of its life have similar fitness outcomes to those that do not give birth, implying that gestation has a minimal cost relative to lactation (Clutton-Brock *et al.*, 1989). The deer are infected with several helminth parasites, with decreased mucosal IgA levels and increased parasite intensity in reproductive females (Albery *et al.*, 2018a; b). Lactation but not gestation results in substantially increased parasite counts, partially reflecting the cost of reproduction for fitness; however, investment in gestation results in decreased mucosal IgA levels (Albery *et al.*, 2018b). Increased mucosal IgA levels also correspond to reduced parasite burden, implying that they are indicative of protective immune responses (Albery *et al.*, 2018b). The deer are not amenable to experimental manipulation, but they are censused regularly, providing detailed information on their survival and reproductive success (Clutton-Brock *et al.*, 1982). In such situations, path analysis can be used to infer links between parasites and their fitness consequences (Pacejka *et al.*, 1998; Stjernman *et al.*, 2004; Brambilla *et al.*, 2015; Leivesley *et al.*, 2019).

Notably, a recent analysis in a wild population of Soay sheep used path analysis to demonstrate that reproduction reduced survival through increased parasite intensity and reduced body weight (Leivesley *et al.*, 2019). Path analysis generally allows inference of likely causal links given one or more *a priori* graphs of interconnected variables (Shipley, 2009). However, it is worth noting that path analysis does not confirm causality any more than other types of statistical analysis: for example, links among variables may belie more complex associations and/or may be confounded with hidden (latent) variables. Path analysis must therefore be used carefully, and in conjunction with specific *a priori* hypotheses and feasible causal pathways (e.g. see Figure 1).

Here, we use path analysis to link reproduction-immunity-parasitism tradeoffs in the Isle of Rum red deer with survival and reproduction in the following year, investigating whether immunity and parasitism are capable of mediating life history tradeoffs, and attempting to separate immune and parasite mediation from direct effects of reproduction acting through alternative mechanisms.

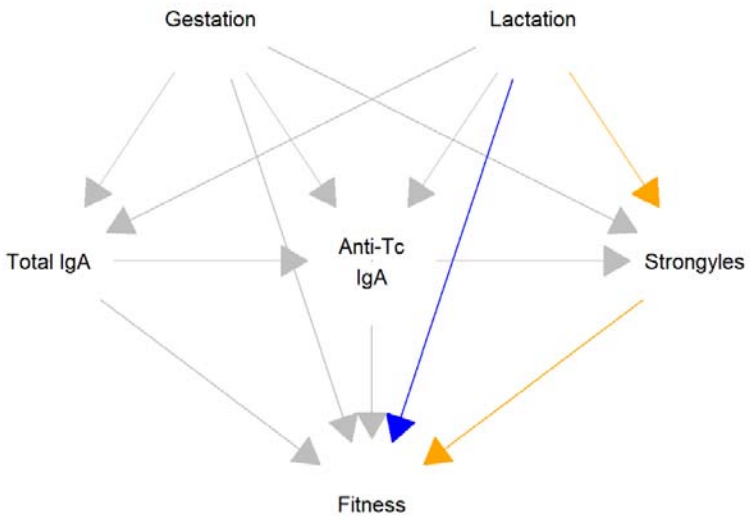

Figure 1: The base Directed Acyclic Graph (DAG) used for all four fitness trait path analyses. The variables are organised into three layers of causality: reproductive allocation (beginning of deer year *t*, top row); immune and parasite variation (deer year t, middle row); and fitness outcomes in the following year (beginning of deer year *t+1*, bottom row). The exception to these timings is survival, for which we investigated effects on samples in both spring in year *t-1* and summer in year *t* on survival to the beginning of year *t+1*. The blue line displays an example direct effect of lactation on fitness; the orange lines display an indirect effect of lactation acting through strongyles. Anti-Tc IgA=anti-*Teladorsagia circumcincta* larval antigen IgA. Not shown: additional links of year, season, and age in years.

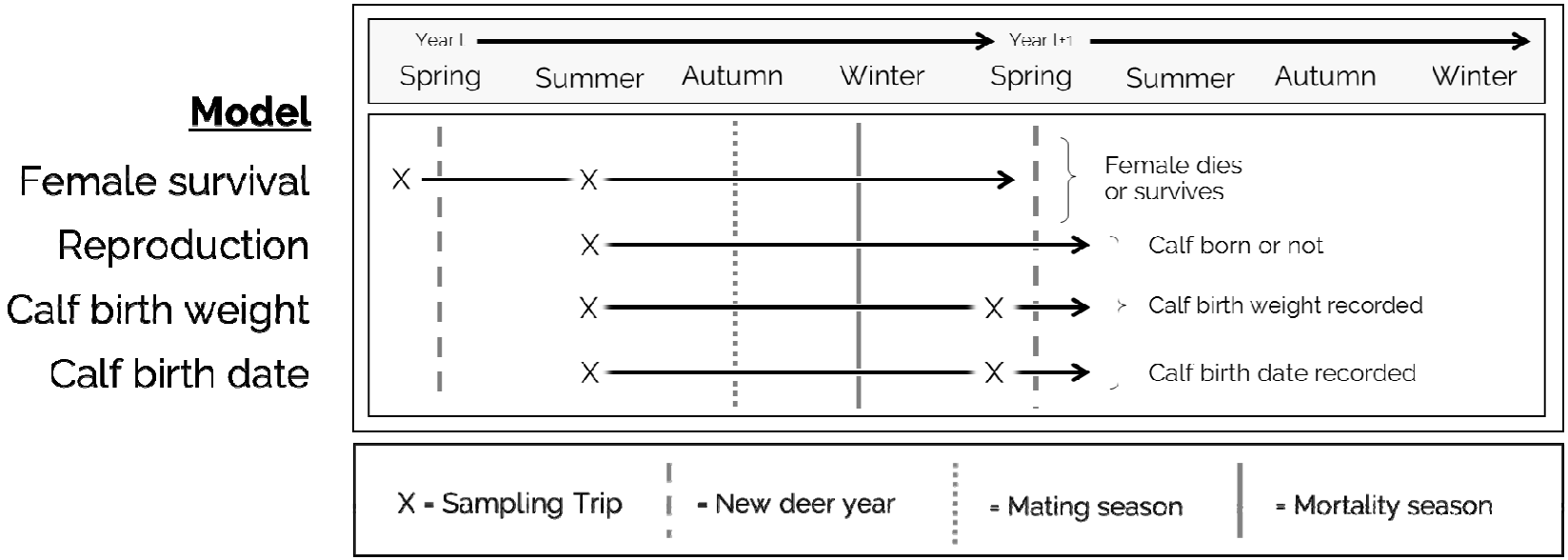

Figure 2: The four models in the context of the red deer reproductive cycle and sampling regime, over an example two-year period. Reproduction begins in spring and summer, at the start of each deer year, and one sampling trip was undertaken each summer (August), after the calving season had finished. Mating occurs in the autumn, and the mortality season begins in winter and lasts until early spring. A second sampling trip occurred each spring (April), after mating and mortality, but before the beginning of the subsequent calving season. The fitness variables investigated were quantified at the start of the subsequent deer year: if a female survived to May 1 the following year she was counted as 1 in the survival analysis, 0 if not, and the presence, weight, and birth date of her calf in the following spring were used as response variables in the remaining three models. The sampling trips included in each model were selected according to feasibility of causal links. For example, females become pregnant in the autumn, so we did not include the spring sampling season in the reproduction model as they would already be pregnant at this point, making it unlikely that parasite counts in April have a direct effect on their probability of having a calf 1-2 months later.

Methods

Study system, sampling, and labwork

The study population is situated in the north block of the Isle of Rum National Nature Reserve (57°N 6°20′W). The deer are entirely wild and unmanaged, and have been monitored continuously since the 1970s (see Clutton-Brock *et al.*, 1982 for an overview of the project). The life history data collected on the population provide high-resolution estimates of individuals' dates of birth and death, reproduction, and familial relationships. The "deer year" begins on May 1st, and the deer begin giving birth ("calving") in May-June, having conceived in the previous autumn (Figure 2). Deer on Rum give birth to a single calf, and do not reproduce every year. During the calving season, we aim to capture and mark as many of the calves born as possible soon after birth, so that they can be monitored for the rest of their lives. Sex and capture weight (to the nearest 100g) are recorded. A proportion of calves (~20%) die within the first few weeks of life, and giving birth to a calf that dies within this period has little cost to the mother in terms of her survival and reproduction probability the following year (Clutton-Brock *et al.*, 1989). In contrast, if a calf survives into the winter, the mother has spent ~6 months lactating to it, expending considerable resources in doing so, and this cost is associated with substantially decreased survival and reproduction probability the following year (Clutton-Brock *et al.*, 1989; Froy *et al.*, 2016).

The sampling regime (Figure 2) has been described previously (Albery *et al.*, 2018b). During early spring (April) and late summer (August), either side of the calving season, we conducted two-week field trips to collect faecal samples from the deer noninvasively. We watched known individuals for defaecation, marked the spot where the droppings landed, and then collected them while minimising disturbance to the deer, generally within an hour. In the evenings, samples were processed and put into Ziploc bags for storage. A subsample was extracted by centrifugation and kept frozen for faecal antibody analysis (Watt *et al.*, 2016; Albery *et al.*, 2018b). Another subsample was kept as anaerobic as possible in a Ziploc bag at 4°C to avoid egg hatching.

In the lab, faecal samples were counted for strongyle nematodes eggs using a salt flotation-centrifugation faecal egg count (FEC), accurate to 1 egg per gram (EPG) within 3 weeks of collection. Strongyles are ubiquitous ruminant parasites that are present at high prevalence in this population and which increase in intensity in lactating individuals (Albery *et al.*, 2018b). Previous studies in this population have also examined the helminths *Elaphostrongylus cervi* and *Fasciola hepatica* (Albery *et al.*, 2018a; b). We chose to examine strongyles but not *E. cervi* or *F. hepatica* for several reasons: we did not want to add too many links to the DAG for reasons of interpretability; strongyles are most expected to have

strong fitness costs (Hoberg *et al.*, 2001) and exhibited the most profound reproductive tradeoff in terms of significance and magnitude (Albery *et al.*, 2018b)a; we did not expect *E. cervi* to have strong fitness effects (Irvine *et al.*, 2006); and *F. hepatica* is present at relatively low prevalence in adult females, preventing it from being fitted easily as an explanatory variable (Albery *et al.*, 2018a). We also carried out antibody detection ELISAs designed to quantify mucosal IgA in sheep (Watt *et al.*, 2016; Albery *et al.*, 2018b). This protocol quantifies both total IgA levels as a measure of general immune investment, and anti-*Teladorsagia circumcincta* IgA levels (anti-Tc IgA) as a specific anti-strongyle measure. *T. circumcincta* is primarily a sheep strongyle, but the anti-Tc IgA assay shows high cross-reactivity with a range of strongyle nematodes including the mouse helminth *Heligmosomoides polygyrus* and thus measures general anti-strongyle responses (Froy *et al.*, in review). The deer are infected with a selection of strongyle nematodes (Irvine *et al.*, 2006), including *Teladorsagia circumcincta* (unpublished data); thus, this measure is used to approximate anti-strongyle immune responses in the deer (Albery *et al.*, 2018b). To control for collection factors which introduce confounding variation in antibody levels we used the residuals from a model including extraction session, time to freezing, and collection day, as in previous studies (Albery *et al.*, 2018b, 2019). We also assayed faecal samples collected in November (e.g. Albery *et al.*, 2018a). However, females exhibited very low strongyle prevalence in the autumn compared with spring and summer, preventing our FEC data from approximating normality and providing little variation to test when fitted as an explanatory variable. Hence, autumn data were excluded from our analyses.

Statistical analysis

To investigate links among our variables we used path analysis using the D-sep method, in which a set of linear models are fitted to the data, with some variables appearing as both response and explanatory variables (Shipley, 2009). Combining the linear models in this way allows identification of potential causal links and mediating variables: namely, does lactation itself have a cost for fitness, or does it act through parasite count?

We created four Directed Acyclic Graphs (DAGs), with four ultimate response variables, each representing a different fitness trait or proxy in the following year (hereafter referred to as “fitness-related traits”; see Figure 2). These measures included two direct fitness measures: overwinter survival (0/1, where 1=survived to May 1 the following year) and reproduction the following year (0/1, where 1=gave birth in the following calving season). We also examined two fitness-associated proxies: calf birth weight (continuous, Gaussian distributed, based on a regression of capture weight on capture age in days) and calf birth date (continuous, Gaussian distributed, based on Julian date that year).

Our analyses used three immune and parasite measures, which included: Total IgA level; Anti-Tc IgA level; Strongyle count per gram of faeces (continuous, log(count+1)-transformed to approximate normality). We included two mutually exclusive binary reproductive categories representing the reproductive cost paid that year (Clutton-Brock *et al.*, 1989): Gestation (gave birth to a calf which died before 1st October that year) and Lactation (gave birth to a calf which survived to 1st October). We also included variables to control for extraneous annual, seasonal, and age-related variation: Year (categorical, with three levels: 2015, 2016, 2017); Season (two levels: Summer, Spring); and Age (continuous, in years).

Each of the four DAGs was composed of four similar models, fitted using the INLA package (Rue and Martino, 2009). in R version 3.5 (R Core Team, 2018). All measures included female ID as a random effect to control for pseudoreplication. First, we composed a set of three "input models", where the response variable was an antibody or parasite measure. The aim of these models was to quantify the association between reproduction and the immune/parasite measures, and to quantify links between these measures themselves.

The models were specified as follows for each of our analyses, with immune/parasite measures in bold and reproduction in italics. These classifications correspond to the middle row and top row of the DAG (Figure 1), respectively. Variables in brackets were included in the models, but are not displayed in the Figure 1 DAG for clarity.

1. **Total IgA** ~ *Gestation* + *Lactation* (+ Age + Season + Year)
2. **Anti-Tc IgA** ~ **Total IgA** + *Gestation* + *Lactation* (+ Age + Season + Year)
3. **Strongyles** ~ **Anti-Tc IgA** + *Gestation* + *Lactation* (+ Age + Season + Year)

Following construction of these input cost models for each DAG, we fitted one "output model" for each DAG, featuring the fitness-related trait as a response variable, and with reproduction, immune and parasite measures as explanatory variables:

4. Fitness trait ~ **Strongyles** + **Anti-Tc IgA** + **Total IgA** + *Gestation* + *Lactation* (+ Age + Year)

Combining these two model sets allowed comparison of the significance and magnitude of different traits' costs for fitness in the following year (Figure 1). Combining the estimates from models 1-3 with the estimates from model 4 allows calculation of the direct and indirect (parasite- or immune-mediated) effects of lactation and gestation on subsequent fitness traits (Figure 1, coloured lines). As an example, we compared the magnitude and credibility intervals of direct lactation effects (effect of lactation in the fitness model [model 4]) with indirect effects (lactation effects on strongyle count [model 3] multiplied by the effects of

strongyle count on fitness [model 4]). We took 1000 posterior draws from each of the lactation-strongyle link and the strongyle-fitness link and multiplied them together, and then derived the 95% credibility intervals for this link. We compared these estimates with those for the direct lactation-fitness link to investigate whether effects of lactation were likely to act independently and/or through strongyle count. The models, fitness measures, and datasets used in each analysis are described in Table 1.

Model Set	Fitness Measure	Definition	Dataset	Samples	Individuals
1	Survival	Female survival the following winter (0/1)	All females (Spring year t-1 and Summer year t)	485	134
2	Reproduction	Female reproduction the following deer year (0/1)	All females (Summer)	223	107
3	Calf birth weight	Calf weight the following deer year (Kg)	Females that reproduced the following May-June (Summer year t and Spring year t)	300	94
4	Parturition date	Date of parturition the following deer year (Days from 1st January)		336	106

Table 1: Descriptions of path analyses and the datasets used.

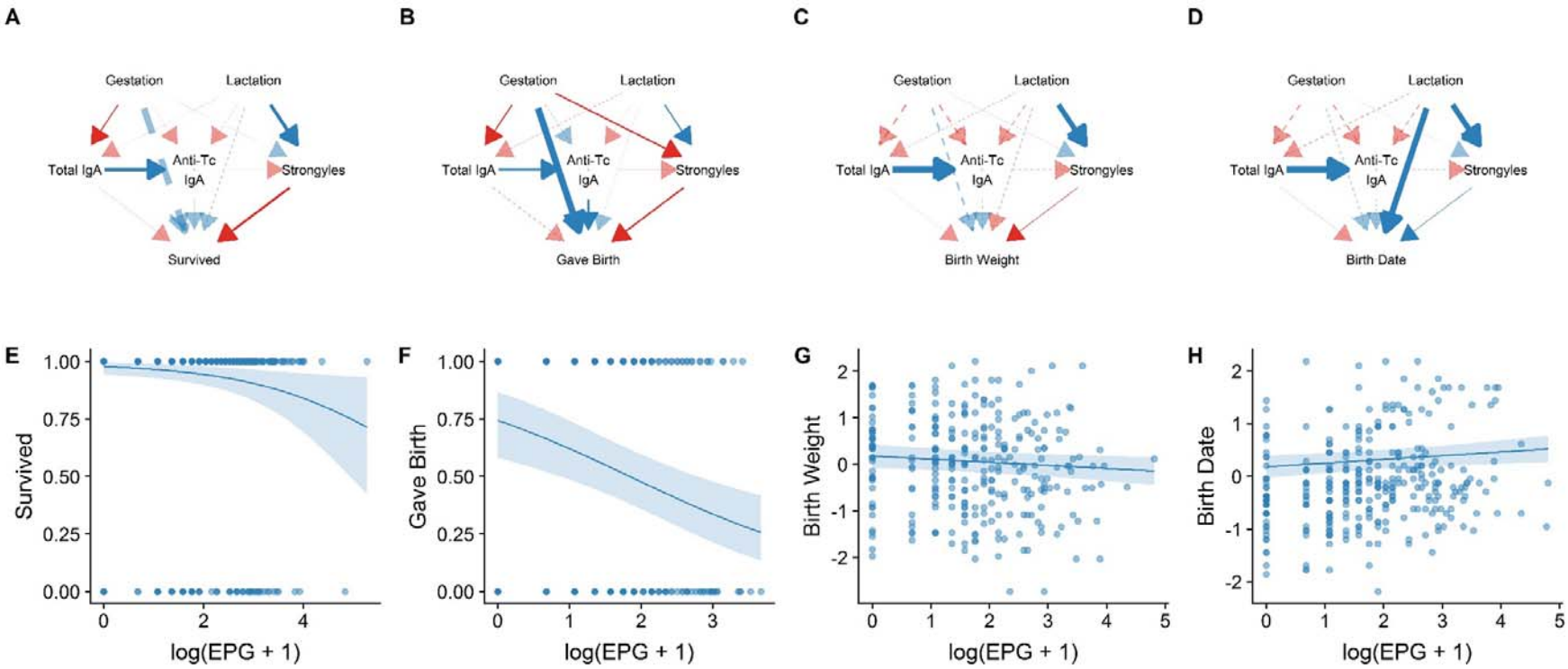

Figure 3: Directed Acyclic Graphs (DAGs) and relationships among parasitism and fitness measures. Results are displayed for all four investigated fitness response variables: overwinter survival (2a,e); reproduction (2b,f); subsequent calf birth weight (2c,g); subsequent parturition date (2d,h). In the DAGs (top row), link colour depends on the direction of the effect (blue=positive, red=negative); link width indicates the magnitude of the effect; and only solid, opaque links are significant (estimates did not overlap with zero). Linear relationships between strongyle count and fitness (bottom row) are based on model estimates for lactating females.

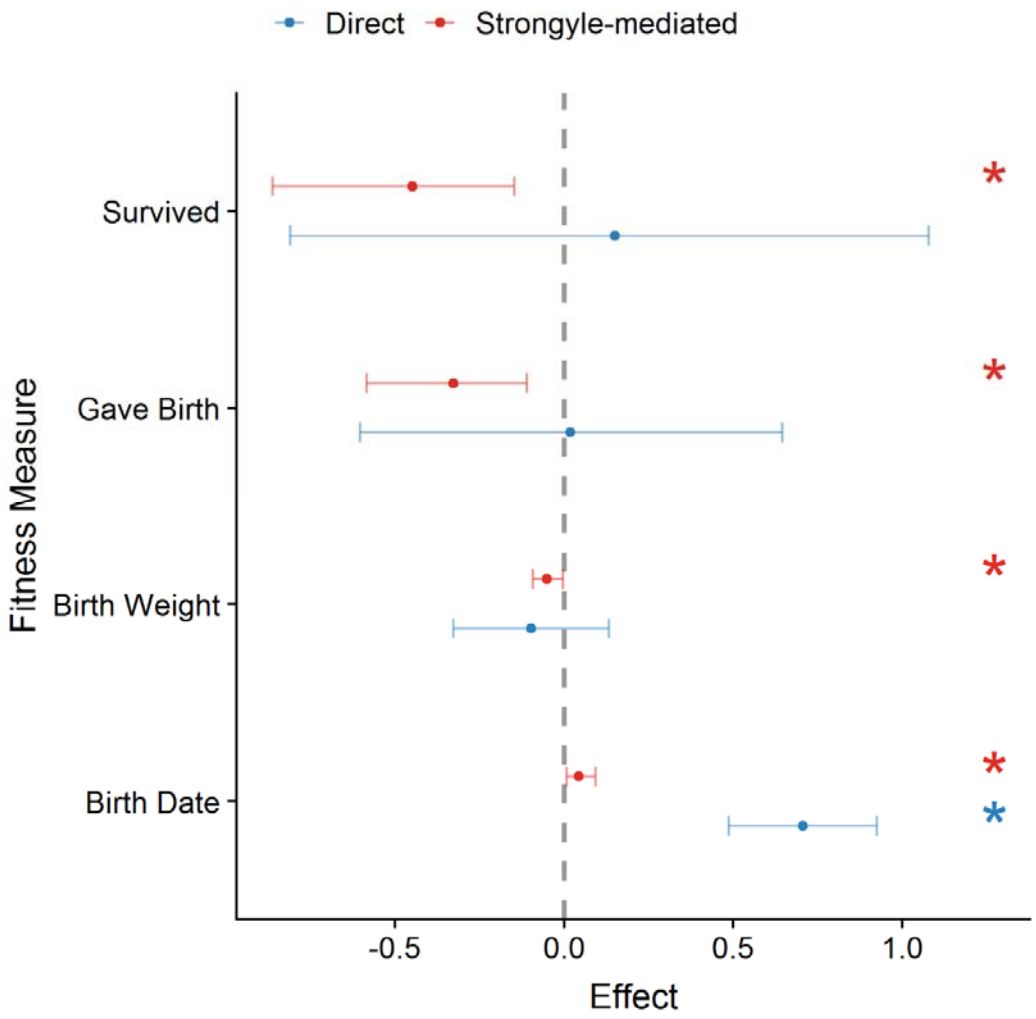

Figure 4: Comparison of direct and indirect (parasite-mediated) effects of lactation on fitness-related traits on the link scale (logistic for survival and reproduction; Gaussian for birth weight and birth date). Points represent mean effect estimates derived from the model posterior distribution; blue corresponds to direct effects, and red corresponds to indirect effects. Parameters with asterisks were significant: i.e., their credibility intervals did not overlap with zero.

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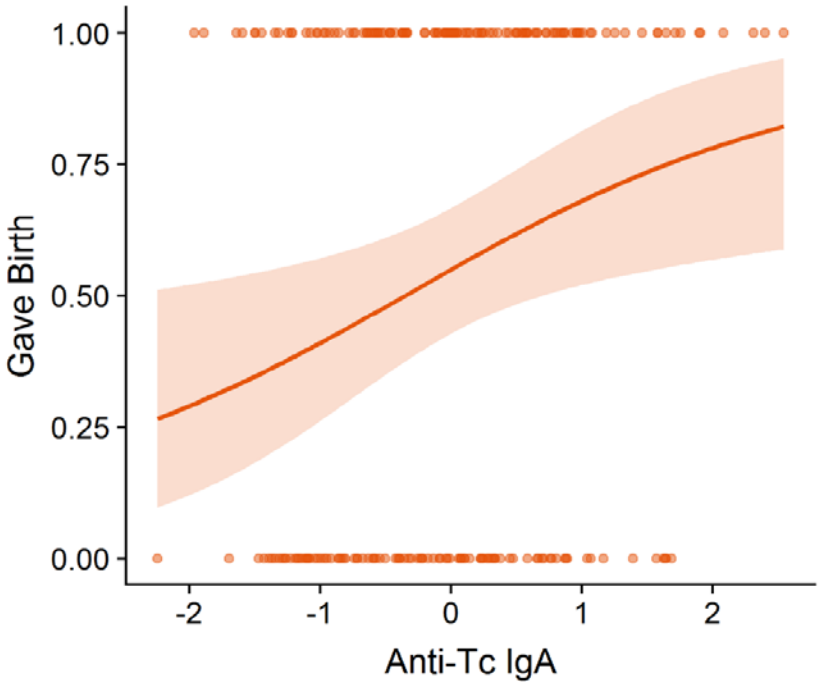

Figure 5: Higher anti-*Teladorsagia circumcincta* IgA was associated with an increased probability of reproducing the following year. Anti-Tc IgA was cube root-transformed and calculated from the residuals of a linear model including collection variables, and was then scaled to have a mean of 0 and a standard deviation of 1. The line represents the output of the reproduction probability model which includes lactation and strongyles as explanatory factors.

Results

Path analyses consistently revealed strong positive associations between lactation and parasitism, and negative associations between parasitism and subsequent fitness-related traits (Figures 3, 4). In contrast, estimates for lactation's association with subsequent fitness itself overlapped with zero for all response variables except parturition date, strongly supporting parasite-mediated reproductive costs for fitness (Figures 3, 4). Below, for each of the four fitness-related response variables, we describe the magnitude of the direct association of parasitism with fitness, the direct association of lactation with fitness, and lactation's association with parasitism multiplied by parasitism's association with fitness. The latter gives an estimate of the indirect effect of lactation on the fitness-related trait acting through strongyle count. For effect sizes we give the mean and 95% credibility intervals (CI). 1 log(EPG+1) increase corresponds to a ~3x increase in strongyle intensity. Full model effect sizes are displayed in the supplementary information (Appendix B, Figure SI1; Table SI1).

Parasitism had a strong association with survival probability despite high survival rates in the population (Figure 3a,e). Females with the lowest counts (0 EPG, 10% of samples) had a survival probability of ~100%, while those with the highest (>25EPG, 7% of samples) had a survival probability of <90% (Figure 3e). Combining this effect of parasitism with the strong positive association between lactation and strongyle count (+0.81, CI 0.64, 0.99) created an indirect cost of lactation acting through strongyle count which corresponds to a decrease in survival probability (Figure 4). Although this effect was highly significant on the link (logistic) scale (Appendix B, Figure SI1, Table SI1), given the high survival rates in the population, at the mean EPG value this lactation-associated increased strongyle intensity would correspond to only a ~2% decrease in survival probability. In contrast, estimates for the direct effect of lactation on survival overlapped widely with zero, and the point estimate was greater than zero, implying that individuals that lactate were slightly more likely to survive when the effects of parasitism were accounted for (Figure 3a,4).

The magnitude of strongyles' association with subsequent reproduction had a similar effect size to its association with overwinter survival (Figure 3b, f; Figure 4; Appendix B, Table SI1; Figure SI1). An increase of 1 log(EPG+1) was associated with a decrease of ~15% probability of reproducing. 0 EPG (17% of samples) corresponded to a ~77% chance of reproducing the following year, and those with >20 EPG (6% of samples) had a reproduction probability of <36% (Figure 3f). The direct effect of lactation on subsequent reproduction was negligible and had very large credibility intervals, as with survival (Figure 4). In addition to the association with parasitism, individuals with higher levels of anti-Tc IgA were considerably more likely to reproduce the following year (Figure 3b; Figure 5). An increase of 1 standard deviation of anti-Tc IgA levels corresponded to an increase of ~10% in the probability of reproducing. Individuals with the lowest anti-Tc IgA levels (< -1 SD units) had a reproduction probability of <50%, compared to >75% for those with the highest levels (>1 SD units FIG). Finally, individuals that paid the cost of gestation were much more likely to reproduce the following year, independently of the effects of antibodies and parasites (Figure 3b).

Calving traits exhibited weaker associations with parasitism than did survival and reproduction, although the results still implied an indirect cost of lactation acting through strongyle count (Figures 3 and 4). The DAG for calf birth weight was similar to that for survival (Figure 3c). An increase of 1 log(EPG+1) corresponded to a slight decrease in calf birth weight the following year (0.07 SD units, or about 86g; Figure 3g). Females with the highest strongyle intensities (>25 EPG) gave birth to calves which were ~400g lighter than those with the lowest intensities (0 EPG), or around 6.24 kg compared to 6.65 kg. As with survival, there was poor support for a direct association between lactation and birth weight

(Figure 4). The estimates for this direct effect were close to zero, and credibility intervals overlapped substantially with zero (Figure 4). Lactation's positive effect on strongyle count once again resulted in a significant negative indirect effect of lactation on subsequent calf birth weight acting through strongyles, but the estimates were very small and nearly overlapped with zero (-0.0438kg, CI -0.111.6, -0.005.6).

In contrast to all other metrics we investigated, there was very strong support for a positive and direct effect of lactation on parturition date the following year: that is, females whose calf survived until the winter were likely to calve later in the following year (~8.5 days later, CI: 5.9, 11.2; Figure 3d; Figure 4), regardless of parasite count. There was a much weaker association between strongyle count and parturition date: an increase of 1 log(EPG+1) produced a delay in calving of ~0.93 days (CI: 0.12, 1.75; Figure 3d,h; Figure 4). Lactation resulted in an increase of 0.7 log(EPG+1). Combining this estimate with the effect of parasitism on birth date gives an estimate for an indirect effect of lactation acting on birth date totalling 0.58 days' delay (CI 0.06, 1.31; Figure 4). Parturition date was thus the only metric examined here for which lactation's direct effect was definitively larger than its indirect effect acting through strongyle count (Figures 3 and 4).

There was a strong positive association between total IgA and anti-Tc IgA, as expected given our previous findings (Albery *et al.*, 2018b; Appendix B, Table SI1, Figure SI1). However, lactation had no significant effect on anti-Tc IgA in our DAGs, showing some discordance with these findings (Figure 3, Appendix B, Table SI1, Figure SI1). This disagreement likely originated from a markedly reduced sample size in our path analysis compared to these previous analyses (Table 1).

Discussion

This study offers strong observational evidence for parasite-dependent mediation of life history tradeoffs in a wild mammal (Williams, 1966), and for broader fitness effects of parasitism, immunity and reproductive allocation. Lactation was associated with higher parasite intensities which translated to reduced survival and reproduction probability in the subsequent year, and among individuals that did reproduce the following year, those with high strongyle counts gave birth later in the year and to smaller calves. Beyond these parasite-mediated effects, lactation had a direct detrimental effect on subsequent parturition date, and increased anti-strongyle IgA levels were associated with increased reproduction probability. Our observation of a direct (parasite-independent) association between lactation and subsequent parturition date suggests that costs of reproduction for subsequent fitness traits can also arise through a combination of direct and parasite-dependent mechanisms. It is likely that the life history tradeoffs exhibited by the Rum red deer population (Clutton-Brock

et al., 1989; Froy *et al.*, 2016) are at least partially mediated by parasites as previously suggested (Albery *et al.*, 2018b), or that parasites and immunity closely correspond to latent condition variables that are responsible for mediating fitness.

Fitness effects of parasites

Lactation's negative association with fitness acted largely through strongyle count for all fitness metrics except parturition date. This may represent a strong parasite-mediated cost, in which pathology and resource allocation associated with increased parasitism are the primary cause of increased overwinter mortality and reduced reproductive fitness in lactating individuals (Clutton-Brock *et al.*, 1989). Allocation of resources to lactation and associated physiological changes may compromise damage repair and resistance mechanisms, leaving lactating females more susceptible to strongyles (Sheldon and Verhulst, 1996; Speakman, 2008). As lactation results in considerably increased forage intake in ungulates (Hamel and Côté, 2008), it is also possible that increased grazing brings resource benefits which fully counteract the direct resource costs of lactation, while intensifying strongyle parasitism (and thereby parasite-associated pathology) through increased exposure (Albery *et al.*, 2018b). High parasite intensity likely causes gut pathology, interfering with nutrient absorption and thereby exacerbating the nutritional scarcity of the winter period, leading to mortality (Gulland, 1992; Pedersen and Greives, 2008; Maublanc *et al.*, 2009), as well as reducing females' ability to achieve the body condition necessary to conceive and carry a calf to term (Albon *et al.*, 1986).

Such fitness costs will exert strong selection pressures on the deer, particularly given the low absolute strongyle counts in the population (median intensity ~= 6 EPG, mean ~= 12 EPG). The relationship between parasitism and reproduction was especially steep (Figure 3b), and the deer will be selected to resist or tolerate infection to avoid such fitness consequences (Graham *et al.*, 2011). Combined with the considerable spatiotemporal variation in parasite exposure in this population (Albery *et al.*, 2019), parasite-mediated reproductive costs could create spatial and temporal variation in the fitness consequences of reproduction. In this case, females that give birth and lactate but then avoid areas with high parasite intensities may avoid the fitness reduction associated with reproduction, which could select for behavioural avoidance of infection (Hutchings *et al.*, 2006; Weinstein *et al.*, 2018). It can be hard to detect survival costs of reproduction in long-lived mammals given their low between-individual variance in survival probability (Hamel *et al.*, 2010). Here we discovered a detectable indirect cost of reproduction for survival – in fact, the link-scale effect sizes of the parasite-mediated lactation effect was similar for both survival and reproduction (Figure 4). In contrast, subsequent calf birth weight and parturition date (both fitness-related proxies rather than absolute fitness traits) had much weaker relationships with parasitism (Figure 3).

Traits that are more tightly linked to fitness such as annual survival and reproduction may be more quickly compromised by parasitism than less-related fitness proxies such as calf birth weight or date, which may have stronger genetic components (Stopher *et al.*, 2012b)s. Previous work in ungulates has demonstrated that immune variation and parasite pressure can maintain variation in life history strategies by affecting reproduction and survival differently, which may likewise be the case in this population (Graham *et al.*, 2010; Garnier *et al.*, 2016). Future investigations could use larger sample sizes collected from more reproductive cycles to determine how the relationships demonstrated here impact calf survival and breeding success.

Alternatively, any latent variable mediating reproduction-fitness tradeoffs may be very well correlated with strongyle count, so that strongyle count assumed the majority of lactation's effect but was not causally responsible for the reduced fitness. For example, hormones can weaken immunity, leading to increased parasitism (Foo *et al.*, 2017), as well as mediating reproductive fitness (Harshman and Zera, 2007). It is possible that fitness-mediating hormones produce a concurrent increase in parasitism in the deer, which then absorbed the variation in fitness attributable to hormonal regulation of reproductive investment in our analysis. Similar arguments could be used for females' body weight, which is a trait generally used to represent broad-strokes condition, and which mediates a life history tradeoff alongside parasite count in Soay sheep (Leivesley *et al.*, 2019). While path analysis has been very useful in revealing interrelationships between the variables studied here, these findings do not prove causality. However, parasites are defined by their ability to cause harm, and strongyles are an important ungulate parasite which cause considerable morbidity and mortality in wild ungulates (Coltman *et al.*, 1999; Hoberg *et al.*, 2001) and which are associated with decreased body condition in this population (Irvine *et al.*, 2006). We therefore have strong reasons to believe that strongyles will impact the health of the deer negatively, and the results of our path analysis conform to this expectation. In addition, the conformation of our findings to the proposed sequence of causality (lactation increases parasitism, which decreases fitness) strengthens our confidence in the inferred importance of strongyles as the mediating factor. Whether or not strongyles are the effectors, our findings nevertheless supports the use of these parasites as a proxy for an individual's health.

The importance of temporal context in reproduction-parasite-fitness relationships

Observational disease ecology studies commonly implicitly assume causal relationships which may in fact be unsupported by the data. In particular, parasitism can act as a response variable reacting to variation in exposure and susceptibility, and/or as an explanatory variable influencing health, resource investment, and ultimately fitness, yet

parasitism is commonly considered from only one of these perspectives. Here, the observed relationship between parasitism and reproduction depended on whether we examined reproduction in the focal year (lactation year t) or in the following year (giving birth in year t+1). Similarly, although we previously reported no relationship of any immune and parasite measures with calf birth date or birth weight in the year of sampling (Albery *et al.*, 2018b), here both fitness measures correlated negatively with strongyle count the previous year. These results demonstrate that fitness metrics quantified at different timepoints can exhibit qualitatively different relationships with parasitism. Therefore, using cross-sectional measures of reproductive investment such as pregnancy in culled animals may be highly misleading, as pregnant individuals may either have become pregnant because they had fewer parasites (Albon *et al.*, 2002; Vandegrift *et al.*, 2008), or may be experiencing higher parasitism due to their increased investment in reproduction (Sheldon and Verhulst, 1996; Knowles *et al.*, 2009; Albery *et al.*, 2018b). This finding supports the virtues of longitudinal studies of known individuals in distinguishing cause from consequence (Clutton-Brock and Sheldon, 2010; Festa-Bianchet *et al.*, 2017).

Among our response variables, only subsequent parturition date was directly associated with lactation the previous year: females that lactated to a surviving calf and then became pregnant gave birth later the following year, regardless of strongyle count. Having raised a calf past its first few months, it is likely that females take longer to regain condition, so they mate later in the year whether or not they are highly parasitized (Albon *et al.*, 1986). As a result, these individuals will calve later the following year (Clements *et al.*, 2011). As lactation and strongyle count are positively correlated, neglecting to include lactation as an explanatory variable in this analysis would substantially exaggerate the perceived importance of parasitism in influencing calving date. When investigating fitness effects of parasitism, it may be advantageous for studies to investigate and control for the influence of life history traits themselves. Future studies in this population could elaborate on these findings by investigating how maternal and calf parasitism correlate and correspond to maternal and calf fitness to investigate transgenerational immunity-parasitism-fitness correlations: a topic that is largely understudied and likely exerts a considerable influence on epidemiological dynamics (Roth *et al.*, 2018).

Interpreting antibody-fitness correlations

Higher anti-Tc IgA levels were associated with substantially increased probability of reproduction the following year (Figure 5). This association is surprising, as it acted independently of any associations between 1) strongyles and probability of reproduction and 2) anti-Tc IgA and strongyles themselves. That is, higher anti-strongyle antibody titres were independently associated with increased reproduction probability rather than acting via

reducing parasitism. Moreover, the association between anti-Tc IgA and reproduction probability received considerably stronger support than any connection between total IgA and any fitness-related traits, implying that the driving mechanisms were specific to anti-strongyle IgA rather than to antibody levels in general. There are several possible explanations for this observation: First, and most probably, anti-Tc IgA may in fact be more strongly correlated with another unmeasured component of individual quality than are strongyles or total IgA. For example, individuals with larger fat reserves may be able to sustain higher anti-Tc IgA levels (Demas *et al.*, 2003) as well as being fitter (Miller *et al.*, 2003; Milenkaya *et al.*, 2015). This possibility may reflect the confounding effects of individual quality in observational studies (van Noordwijk and de Jong, 1986). However, it is surprising that this trend was evident for anti-Tc IgA rather than total IgA, as we expected the latter to be a better general indicator of health/condition than parasite-specific IgA. Alternatively, anti-Tc IgA may be protective against other, unmeasured parasites, reducing their impact on fitness, or it may reduce or repair damage from strongyles rather than acting through reducing intensity. That is, anti-Tc IgA may be an indicator of tolerance as well as of resistance (Råberg *et al.*, 2009b; Graham *et al.*, 2011). Regardless of the cause, this observation supports the value of examining the fitness consequences of immune variation itself, rather than examining parasitism alone (Graham *et al.*, 2011), and of studying parasite-specific antibodies in general (Garnier and Graham, 2014). In particular, path analysis presents a convenient method for contrasting the relative importance of immunity and parasites and their interrelationships in influencing fitness, particularly when life history allocation itself may have a cost.

A previous analysis demonstrated costs of reproduction for immunity as well as parasitism, where investment in lactation was associated with lower total IgA and anti-Tc IgA levels, and gestation with lower total IgA levels (Albery *et al.*, 2018b). Although our estimates fell within the estimates for this previous analysis (SIFig 1), our estimates of lactation's association with total IgA and anti-Tc IgA were weaker, and overlapped marginally with zero in all cases. This weaker association likely emerged from the reduced sample size in the present analysis compared to our previous analysis, due to our exclusion of the autumn sampling season. As a result, although anti-Tc IgA was associated with increased probability of reproduction the following year, we were unable to identify this as part of a life history tradeoff. It was not possible to transform the autumn parasite counts to conform to normality, and the range of strongyle counts gave very little variation to test, precluding our ability to include this season in the analysis. The detection of tradeoffs was thus dependent on the power of the analysis used, and apparently on the time of year at which the tradeoff is analysed; it is possible that further sampling of the population will allow us to identify these tradeoff mechanisms using

analyses with increased power. As we did not uncover any other fitness benefits of immunity, parasites themselves may be a more reliable general indicator of a deer's health than the immune responses to these parasites.

This study offers long-awaited evidence for parasite-dependent mediation of life history tradeoffs in a wild animal population (Williams, 1966). We demonstrate that reproductive costs for fitness metrics may originate largely from parasites, and potentially through alternative mechanisms for the case of parturition date. We suggest that more studies should simultaneously examine the fitness consequences of reproduction, parasites, and immunity in tandem.

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Supplementary Information

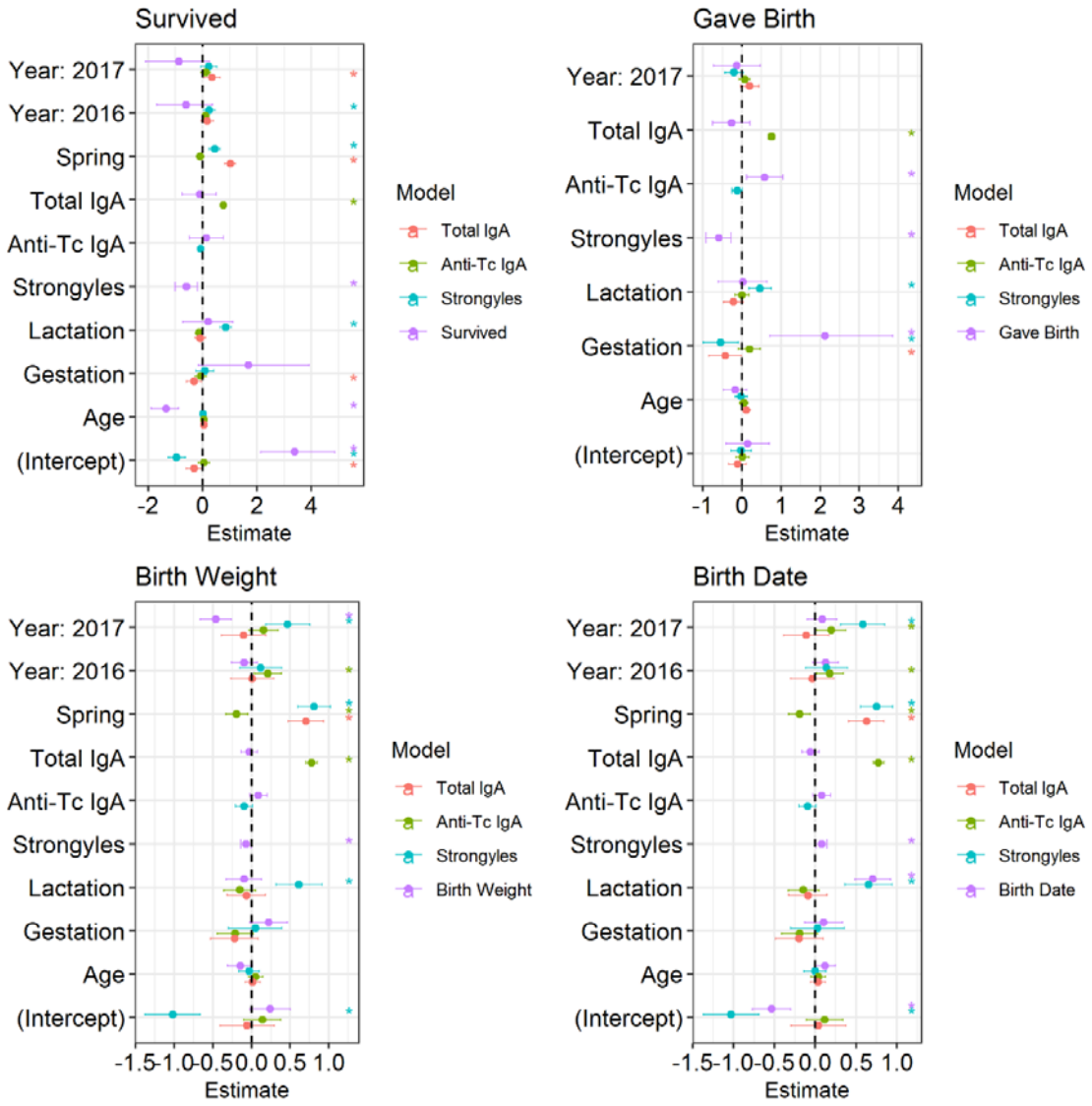

Figure SI1: Model outputs for the component GLMMs of all four path analyses. Colours correspond to different response variables; variables on the y axis correspond to explanatory variables in each component model. Points denote mean estimates from the posterior distribution of effect sizes from the component INLA model. Error bars denote 95% credibility intervals for the distribution. Where these intervals did not overlap with 0, the effect was taken to be significant. These effects are marked with an asterisk. Notably, lactation was positively associated with strongyles (blue estimates) and strongyles were negatively associated with all fitness-related traits (purple estimates).

Survival Path Analysis				
Model	Variable	Estimate	Lower CI	Upper CI
Total IgA	Intercept	-0.3081	-0.609	-0.0075
	Gestation	-0.3182	-0.5999	-0.0367
	Lactation	-0.0912	-0.2842	0.1017
	Age	0.047	-0.0273	0.1212
	Spring	1.0171	0.8138	1.2202
	Year: 2016	0.1765	-0.0441	0.397
	Year: 2017	0.34	0.0436	0.6363
Anti-Tc IgA	Intercept	0.0571	-0.149	0.263
	Total IgA	0.767	0.7095	0.8243
	Gestation	-0.0705	-0.2829	0.1417
	Lactation	-0.1298	-0.2625	0.0027
	Age	0.0538	-0.0285	0.1362
	Spring	-0.0872	-0.2234	0.0491
	Year: 2016	0.109	-0.0265	0.2443
	Year: 2017	0.1151	-0.0715	0.3016
Strongyle Count	Intercept	-0.9499	-1.2653	-0.6347
	Gestation	0.083	-0.2426	0.4084
	Lactation	0.8496	0.6416	1.0569
	Anti-Tc IgA	-0.0785	-0.1675	0.0105
	Age	0.0192	-0.1007	0.1382
	Spring	0.4485	0.2474	0.6495
	Year: 2016	0.2491	0.0375	0.4607
	Year: 2017	0.2247	-0.0654	0.5148

Survival	Intercept	3.3842	2.1424	4.8575
	Gestation	1.6766	-0.1589	3.9344
	Lactation	0.2061	-0.7305	1.1135
	Anti-Tc IgA	0.1306	-0.4932	0.7636
	Total IgA	-0.1087	-0.7467	0.5113
	Strongyles	-0.5896	-1.0085	-0.1937
	Age	-1.3464	-1.8883	-0.8885
	Year: 2016	-0.6102	-1.6816	0.364
	Year: 2017	-0.8665	-2.1049	0.2666
Reproduction Path Analysis				
Model	Variable	Estimate	Lower CI	Upper CI
Total IgA	Intercept	-0.1121	-0.3346	0.1102
	Gestation	-0.4279	-0.8417	-0.0145
	Lactation	-0.2216	-0.471	0.0276
	Age	0.1051	-0.0108	0.2209
	Year: 2017	0.1972	-0.0388	0.4329
Anti-Tc IgA	Intercept	0.0148	-0.1561	0.1853
	Total IgA	0.755	0.6747	0.8351
	Gestation	0.1878	-0.0955	0.47
	Lactation	-0.0021	-0.1816	0.1771
	Age	0.047	-0.0583	0.1522
	Year: 2017	0.0692	-0.07	0.208
Strongyle Count	Intercept	-0.0191	-0.2778	0.2403
	Gestation	-0.5432	-0.9927	-0.0939
	Lactation	0.4618	0.1793	0.7444
	Anti-Tc	-0.1244	-0.2597	0.0106

	IgA			
	Age	-0.0228	-0.1747	0.1275
	Year: 2017	-0.2038	-0.4353	0.0286
Gave Birth	Intercept	0.1424	-0.4085	0.6949
	Gestation	2.122	0.7159	3.8491
	Lactation	0.0188	-0.6041	0.6448
	Anti-Tc IgA	0.5761	0.1144	1.0549
	Total IgA	-0.2661	-0.7449	0.2034
	Strongyles	-0.5885	-0.9166	-0.2737
	Age	-0.1704	-0.4716	0.1247
	Year: 2017	-0.1285	-0.7295	0.4681
Calf Birth Weight Path Analysis				
Model	Variable	Estimate	Lower CI	Upper CI
Total IgA	Intercept	-0.0559	-0.4059	0.2937
	Gestation	-0.2193	-0.5256	0.0866
	Lactation	-0.0667	-0.3142	0.1807
	Age	0.0129	-0.0855	0.1113
	Spring	0.7025	0.4721	0.9326
	Year: 2016	0.0116	-0.2658	0.2888
	Year: 2017	-0.1004	-0.387	0.186
Anti-Tc IgA	Intercept	0.1405	-0.0992	0.3799
	Total IgA	0.7789	0.7074	0.8506
	Gestation	-0.2126	-0.4438	0.018
	Lactation	-0.1524	-0.3547	0.0498
	Age	0.0525	-0.043	0.148
	Spring	-0.1912	-0.3348	-0.0479

	Year: 2016	0.2109	0.0333	0.388
	Year: 2017	0.154	-0.0338	0.3416
Strongyle Count	Intercept	-1.0168	-1.3738	-0.6603
	Gestation	0.0489	-0.2936	0.3925
	Lactation	0.6138	0.3177	0.9118
	Anti-Tc IgA	-0.0962	-0.2046	0.0121
	Age	-0.0306	-0.162	0.1018
	Spring	0.8109	0.5991	1.0222
	Year: 2016	0.1204	-0.15	0.3911
	Year: 2017	0.4678	0.1848	0.7503
Calf Birth Weight	Intercept	0.2411	-0.0236	0.5057
	Gestation	0.2213	-0.023	0.4654
	Lactation	-0.0972	-0.3267	0.1319
	Anti-Tc IgA	0.0909	-0.0243	0.2057
	Total IgA	-0.0278	-0.1342	0.0788
	Strongyles	-0.0715	-0.1404	-0.0028
	Age	-0.1467	-0.3092	0.0156
	Year: 2016	-0.0935	-0.2613	0.0742
	Year: 2017	-0.461	-0.6596	-0.2627
Calf Birth Date Path Analysis				
Model	Variable	Estimate	Lower CI	Upper CI
Total IgA	Intercept	0.0381	-0.2974	0.3734
	Gestation	-0.1961	-0.4888	0.0963

	Lactation	-0.0889	-0.3236	0.1457
	Age	0.0342	-0.0598	0.1282
	Spring	0.6269	0.409	0.8447
	Year: 2016	-0.0365	-0.302	0.2289
	Year: 2017	-0.1125	-0.3907	0.1654
Anti-Tc IgA	Intercept	0.1135	-0.112	0.3389
	Total IgA	0.7769	0.7097	0.8441
	Gestation	-0.1909	-0.4103	0.028
	Lactation	-0.1438	-0.3309	0.0431
	Age	0.0369	-0.0546	0.1286
	Spring	-0.1948	-0.3278	-0.0619
	Year: 2016	0.1799	0.0144	0.3452
	Year: 2017	0.1978	0.0202	0.3753
Strongyle Count	Intercept	-1.0305	-1.3734	-0.6883
	Gestation	0.0273	-0.3045	0.3598
	Lactation	0.6512	0.3645	0.9407
	Anti-Tc IgA	-0.0935	-0.197	0.0098
	Age	-0.0031	-0.1375	0.1319
	Spring	0.7492	0.5533	0.9449
	Year: 2016	0.14	-0.1144	0.3951
	Year: 2017	0.5809	0.3106	0.8508
Calf Birth Date	Intercept	-0.5344	-0.7683	-0.3033
	Gestation	0.1028	-0.1294	0.3341
	Lactation	0.7067	0.4869	0.9255
	Anti-Tc IgA	0.08	-0.0294	0.1894

	Total IgA	-0.0593	-0.1619	0.0429
	Strongyles	0.0772	0.0102	0.1444
	Age	0.1201	-0.008	0.249
	Year: 2016	0.1251	-0.0301	0.2797
	Year: 2017	0.085	-0.0971	0.2668

Table SI1: effect sizes and 95% credibility intervals for the component GLMMs of each path analysis