Fitness Costs of Parasites Explain Multiple Life-History Trade-Offs in a Wild Mammal

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ABSTRACT: Reproduction in wild animals can divert limited resources away from immune defense, resulting in increased parasite burdens. A long-standing prediction of life-history theory states that these parasites can harm the reproductive individual, reducing its subsequent survival and fecundity, producing reproduction-fitness trade-offs. Here, we examined associations among reproductive allocation, immunity, parasitism, and subsequent survival and fecundity in a wild population of individually identified red deer (*Cervus elaphus*). Using path analysis, we investigated whether costs of lactation in terms of downstream survival and fecundity were mediated by changes in strongyle nematode count and mucosal antibody levels. Lactating females exhibited increased parasite counts, which were in turn associated with substantially decreased fitness in the following year in terms of overwinter survival, fecundity, subsequent calf weight, and parturition date. This study offers observational evidence for parasite regulation of multiple life-history trade-offs, supporting the role of parasites as an important mediating factor in wild mammal populations.

Keywords: wild mammal, fitness costs, helminths, survival, reproduction, path analysis.

Introduction

A fundamental tenet of life-history theory states that reproduction should reduce subsequent survival and fecundity (Williams 1966; Stearns 1989). While evidence for such trade-offs is widespread, the mechanisms behind them remain poorly understood. One hypothesized mechanism is that reproductive costs act through parasites, where increased reproductive allocation diverts limited resources away from immune defense, resulting in increased parasite burdens, which reduce subsequent survival and fe-

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cundity (Sheldon and Verhulst 1996; Harshman and Zera 2007). Parasite mediation of life-history trade-offs involves two necessary components: that reproduction increases parasitism and that these 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. First, reproduction is often associated with weaker or altered immune allocation (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). Second, increased parasitism is often associated with decreased subsequent probability of survival (Coltman et al. 1999; Leivesley et al. 2019) or reproduction (Albon et al. 2002; Vandegrift et al. 2008; Hughes et al. 2009). Despite evidence for one or other of these processes in isolation, reproduction-associated increases in parasitism have rarely been linked to downstream survival and fecundity consequences within the same study system to provide full support for parasite mediation of life-history trade-offs.

While it is true that reproduction, immunity, and parasites all compete for host resources, mechanisms governing life-history trade-offs are hypothesized to occur in a temporal sequence rather than occurring simultaneously (fig. 1). First, reproduction diverts resources away from immunity, reducing immune allocation (Sheldon and Verhulst 1996). Resultant weaker immunity, plus potentially increased exposure associated with altered behavior of reproductive individuals (e.g., through increased foraging), can then result in higher parasite burden (Knowles et al. 2009; Albery et al. 2020b). Finally, subsequent survival and fecundity is reduced by damage from parasites (Harshman and Zera 2007; Graham et al. 2011). This combination of mechanisms 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
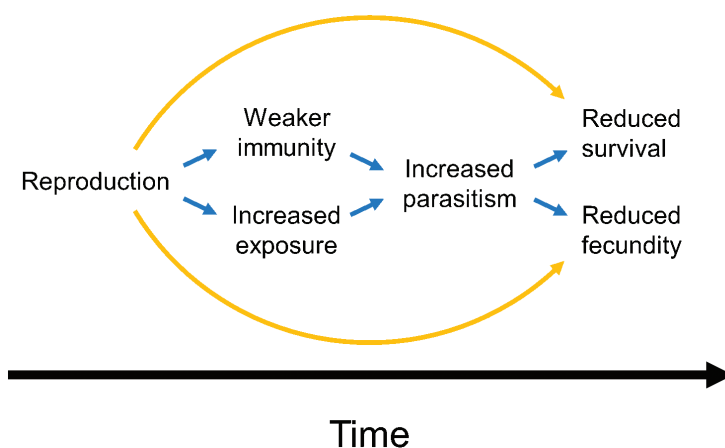
Figure 1: The hypothesized mechanism for parasite-dependent mediation of life-history trade-offs. Blue (interior) arrows denote indirect, parasite-mediated costs of reproduction for downstream survival and fecundity, while the orange (exterior) arrows denote direct costs through resource allocation, hormonal regulation, or similar mechanisms.

and Zera 2007; Speakman 2008; fig. 1). This causal sequence is important, because parasites' observed relationship with life-history traits can depend on whether a preceding, contemporary, or subsequent trait is chosen for examination.

Many studies examining reproduction-immunity-parasitism interrelationships have been carried out in birds, often using experiments in which reproductive effort is artificially increased by manipulating clutch sizes (Knowles et al. 2009). Such manipulations are not possible in many mammal species, and thus most of our knowledge of these trade-offs is based on observational studies. In observational contexts or in concert with experiments, 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 observationally that reproduction reduced survival through increased parasite count and reduced body weight (Leivesley et al. 2019), but it did not examine impacts on subsequent reproductive traits.

The wild red deer (*Cervus elaphus*) on the Isle of Rum provide a classic example of a life-history trade-off under natural conditions: female deer that invest in lactation have reduced survival probability and fecundity in the subsequent year compared with nonlactating females (Clutton-Brock et al. 1989; Froy et al. 2016). Females that give birth to a calf that dies within the first few months of its life have survival and fecundity the following year similar 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; egg counts and mucosal antibody (immunoglobulin A [IgA]) levels are measured via noninvasive collection of fecal samples (Albery et al. 2018, 2020b). A previous study demonstrated

decreased mucosal IgA among females that gestate a calf (Albery et al. 2020b). Lactation (but not gestation) is associated with increased parasite counts, partially mirroring the previously observed costs of lactation for subsequent survival and fecundity (Clutton-Brock et al. 1989; Froy et al. 2016). Together, these studies demonstrate a cost of reproduction for subsequent survival, fecundity, parasitism, and immunity, respectively, in this population. However, we have yet to establish the degree to which subsequent fitness costs of reproduction can be explained by changes in parasitism.

Here, we use path analysis to link reproduction-immunity-parasitism trade-offs in the Isle of Rum red deer with survival and reproductive traits in the following year, investigating whether immunity and parasitism are capable of mediating life-history trade-offs and attempting to separate immune and parasite mediation from direct effects of reproduction acting through alternative mechanisms. We expected that the substantially increased parasite counts associated with lactation would be associated with decreased subsequent survival, fecundity, parturition date, and calf birth weight, such that parasites provide a mechanistic explanation for the costs of lactation seen in this system.

Methods

Study System, Sampling, and Laboratory Work

The study population lives 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 they have been monitored continuously since the 1970s (for an overview of the project, see Clutton-Brock et al. 1982). 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 1, and the deer give birth ("calving") in May and June, having conceived in the previous autumn (fig. 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 100 g) are recorded. Approximately 20% of calves 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 fecundity and survival probability the following year (Clutton-Brock et al. 1989; Froy et al. 2016).

During early spring (April) and late summer (August)—either side of the calving season—we conducted 2-week field trips to collect fecal samples from the deer noninvasively. These trips were conducted separately from the censuses and calving and mortality searches that facilitated the life-history data collection. Sampling was undertaken across five trips from 2016 to 2018, with 701 fecal samples collected in total; see table 1 for details on data sets. We

watched known individuals for defecation, marked the spot where the droppings landed, and then collected them while minimizing disturbance to the deer, generally within an hour. In the evening, samples were aliquoted and processed. A subsample was extracted by centrifugation and frozen at −20°C for fecal antibody analysis (Watt et al. 2016; Albery et al. 2020b). Another subsample was kept as anaerobic as possible in a resealable plastic bag at 4°C to avoid egg hatching.

Within 3 weeks of collection, the fecal samples stored at 4°C were counted for the eggs of gastrointestinal strongyle nematodes using a salt flotation-centrifugation fecal egg count (FEC), accurate to 1 egg per gram (EPG; Kenyon et al. 2013; Taylor et al. 2015). This followed previously established methodology for the study population (Albery et al. 2018, 2020b). Strongyles are ubiquitous ruminant parasites that are present at high prevalence in the study population, exhibiting greater-intensity infections in lactating individuals (Albery et al. 2020b). Previous studies in this population have also examined the nematode parasite *Elaphostrongylus cervi* and the trematode parasite *Fasciola hepatica* (Albery et al. 2018, 2020b). We chose to analyze strongyles but not *E. cervi* or *F. hepatica* for several reasons: we did not want to add too many links to the analysis for reasons of interpretability; strongyles are expected to inflict the greatest costs (Hoberg et al.
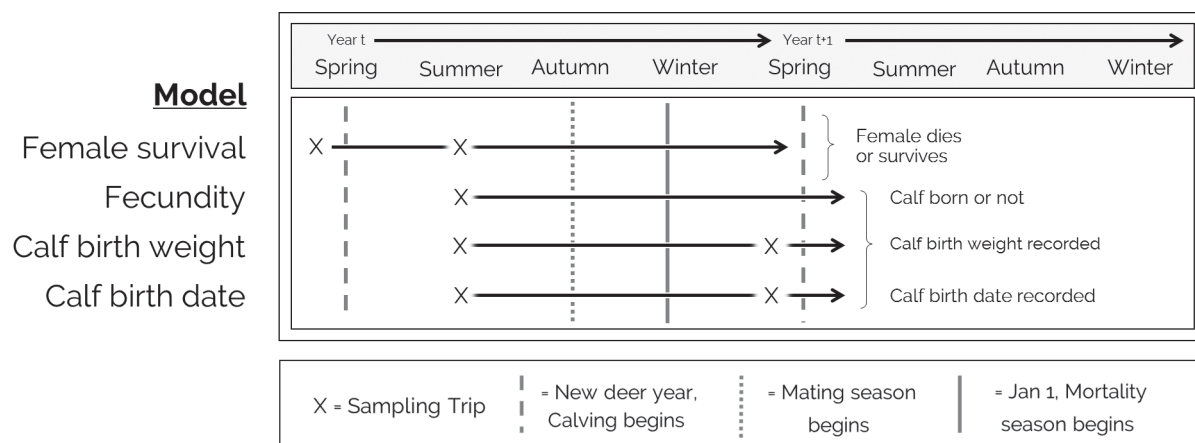

Figure 2: The four models fitted in this study in the context of the red deer reproductive cycle and sampling regime over an example 2-year period. Reproduction begins in spring and summer, at the start of each deer year, and one sampling trip was undertaken each summer (August), after the calving season 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 and 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. NB: The parasitological and immunological data were derived through specific fecal sampling trips (represented by X marks), while survival and calving data were collected through routine censusing operations separate from the fecal sampling trips, providing data that we then linked with the immunoparasitology to infer fitness consequences.

Table 1: Descriptions of path analyses and the data sets used

Model set	Fitness measure	Definition	Data set	Samples	Individuals
1	Survival	Female survival to end of year $t + 1$ (0/1)	All females (spring and summer year t)	482	134
2	Fecundity	Female reproduction in year $t + 1$ (0/1)	All females (summer year t)	221	107
3	Calf birth weight	Calf weight the year $t + 1$ (kg)	Females that reproduced the following May–June (summer year t and spring year t)	298	98
4	Parturition date	Date of parturition year $t + 1$ (days from January 1)	Females that reproduced the following May–June (summer year t and spring year t)	329	104

2001) and exhibited the most profound changes associated with reproduction in terms of significance and effect size (Albery et al. 2020b); 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. 2018).

We carried out antibody detection enzyme-linked immunosorbent assays designed to quantify mucosal IgA in sheep using the fecal extractions stored at −20°C (Watt et al. 2016; Albery et al. 2020b). This protocol quantifies both total IgA levels as a measure of general immune investment and anti–*Teladorsagia circumcincta* IgA (anti-Tc IgA) levels as a specific antistrongyle measure. *Teladorsagia 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* (Froy et al. 2019). The deer are infected with a selection of strongyle nematodes (Irvine et al. 2006), including *T. circumcincta* (S. Ebdon and G. Albery, unpublished data); thus, anti-Tc IgA is used to approximate antistrongyle immune responses in the deer (Albery et al. 2020b). To control for collection factors that 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. 2019, 2020b).

We also assayed fecal samples collected in November (Albery et al. 2018). 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-separation 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.

We created four directed acyclic graphs (DAGs), each examining a different fitness-related trait measured in the year following measurement of parasite burden (see fig. 2). These measures included two fitness components: the female's overwinter survival (0/1, where 1 indicates survival to May 1 in year $t + 1$) and fecundity in year $t + 1$ (0/1, where 1 indicates giving birth in the following calving season). We also examined two fitness-associated calving traits: the birth weight of a female's calf in year $t + 1$ (continuous, Gaussian distributed, based on a regression of capture weight on capture age in days) and parturition date in year $t + 1$ (continuous, Gaussian distributed, based on Julian date that year).

Our analyses used three immune and parasite measures: total IgA level, anti-Tc IgA level, and strongyle count per gram of feces (continuous, log(count + 1) transformed to approximate normality). All were examined using a Gaussian error distribution. We included two mutually exclusive binary reproductive categories representing the reproductive effort made in year t (Clutton-Brock et al. 1989): gestation$_t$ (gave birth to a calf that died before October 1 that year) and gestation$_t$ + lactation$_t$ (gave birth to a calf that survived to October 1; hereafter referred to as simply "lactation," as all individuals that lactated must have also undergone the cost of gestation). We also included variables to control for 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 2020). All measures included female identity as a random effect to control for pseudoreplication. First, we ran 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 in year t and the immune/parasite measures and to quantify links between these measures.

The models were specified as follows for each of our analyses, with immune/parasite measures in boldface and reproductive traits in italics (variables in parentheses were included in the models but are not displayed in the DAGs for clarity): model 1—**total IgA**$_t$ ~ *gestation*$_t$ + *lactation*$_t$ (+ age + season + year); model 2—**anti-Tc IgA**$_t$ ~ **total IgA**$_t$ + *gestation*$_t$ + *lactation*$_t$ (+ age + season + year); model 3—**strongyles**$_t$ ~ **anti-Tc IgA**$_t$ + *gestation*$_t$ + *lactation*$_t$ (+ age + season + year); model 4—*fitness-related trait*$_{t+1}$ ~ **strongyles**$_t$ + **anti-Tc IgA**$_t$ + **total IgA**$_t$ + *gestation*$_t$ + *lactation*$_t$ (+ age + year).

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 survival and reproduction in year $t + 1$ (fig. 1). As an example, we compared the magnitude and 95% credibility intervals (CIs) of direct lactation effects (effect of lactation in the fitness-related trait model [model 4]) with indirect effects (lactation effects on strongyle count [model 3] multiplied by the effects of strongyle count on the fitness-related trait [model 4]). We took 1,000 posterior draws from both the lactation-strongyle link and the strongyle-fitness trait link and multiplied them together, and we then derived the 95% CIs for this link. We compared these estimates with those for the direct lactation-fitness trait link to investigate whether effects of lactation were likely to act independently and/or through strongyle count. The models, fitness measures, and data sets used in each analysis are described in table 1.

Results

Path analyses consistently revealed strong positive associations between lactation and parasite count and negative associations between parasite count and subsequent survival and fecundity (figs. 3–5). In contrast, estimates for lactation's direct association with subsequent survival and fecundity overlapped with zero for all response variables except parturition date, supporting parasite-mediated costs of reproduction (figs. 3–5). Below, for each of the four fitness-related response variables, we describe the magnitude of the direct association with parasitism and with lactation, and lactation's association with parasitism multiplied by parasitism's association with the subsequent fitness-related trait (i.e., survival or fecundity). 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% CI. An increase of 1 log(EPG + 1) corre-

sponds approximately to a threefold increase in strongyle count. Full model effect sizes are displayed in figure SI1 and table SI1 (both available online).

Parasite count had a strong association with subsequent survival probability despite high survival rates in the population (figs. 3A, 4A). Females with the lowest counts (0 EPG; 10% of samples) had a survival probability of ~100%, while those with the highest (>25 EPG; 7% of samples) had a survival probability of <90% (fig. 4A). Lactation was associated with increased strongyle count (+0.85; 95% CI: 0.64–0.99), so that a substantial cost of lactation for survival acted through parasitism (fig. 5). Although this effect was highly significant on the link (logistic) scale (fig. SI1; table SI1), given the high survival rates in the population, at the mean EPG value this lactation-associated increased strongyle parasitism 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 (figs. 3A, 5).

Strongyles' association with subsequent reproduction had an effect size similar to its association with overwinter survival (figs. 3B, 4B, 5, SI1; table SI1). An increase of 1 log(EPG + 1) was associated with a decrease of ~15% probability of reproducing. Zero 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% (fig. 4B). The direct effect of lactation on subsequent fecundity was negligible and had very large CIs, as with survival (fig. 5). In addition to the association with parasite count, individuals with higher levels of anti-Tc IgA were more likely to reproduce the following year (figs. 3B, 6). An increase of 1 standard deviation in anti-Tc IgA levels corresponded to an increase of ~10% in the probability of reproducing. Individuals with the lowest anti-Tc IgA levels (less than −1 SD unit) had a reproduction probability of <50%, compared with >75% for those with the highest levels (>1 SD unit; fig. 6). 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 (fig. 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 (figs. 3–5). The DAG for calf birth weight was similar to that for survival (fig. 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 86 g; fig. 4C). Females with the highest strongyle intensities (>25 EPG) gave birth to calves that were ~400 g lighter than those with the lowest intensities (0 EPG), or
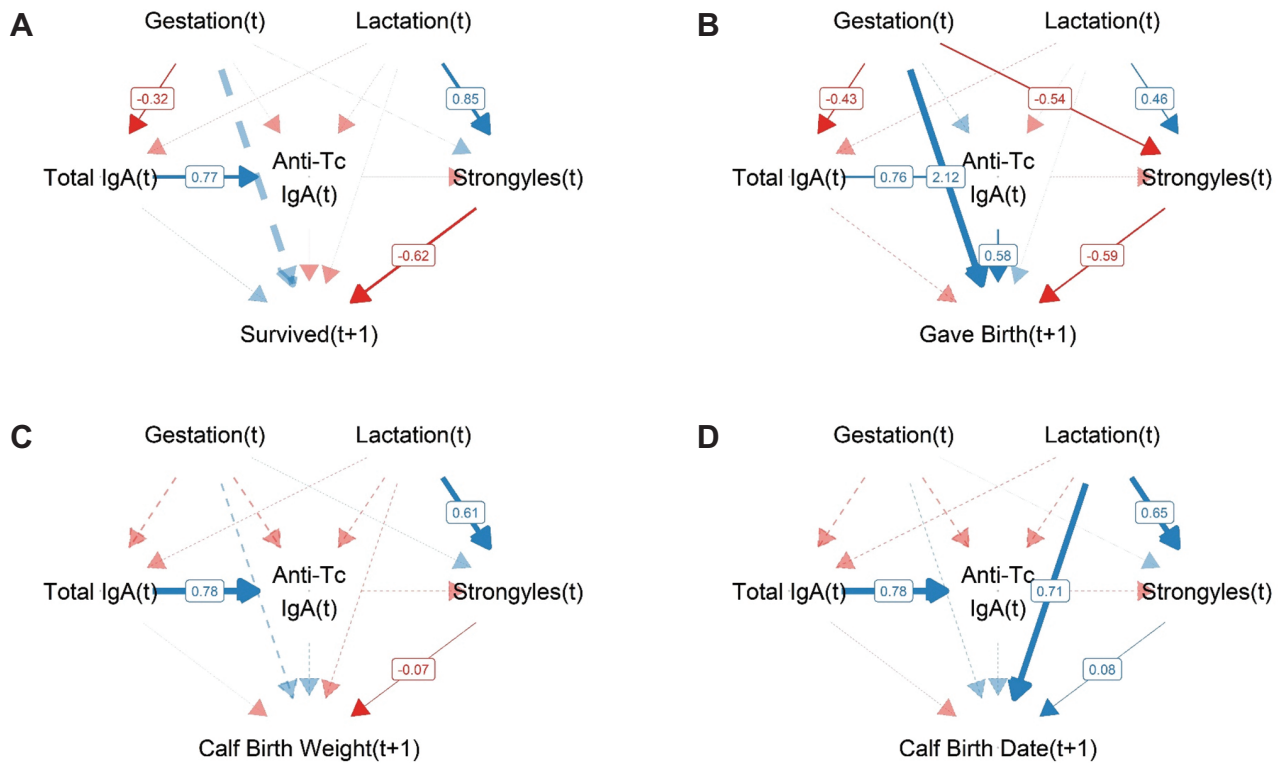
Figure 3: Directed acyclic graphs (DAGs). Results are displayed for all four investigated female fitness-related response variables: overwinter survival (*A*), reproduction the following year (*B*), subsequent calf birth weight (*C*), and subsequent parturition date (*D*). Link color depends on the direction 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). Labels denote the link-scale effect sizes (slopes) for the significant effects, derived from generalized linear mixed models (full model effects are displayed in fig. SI1). Anti-Tc IgA = anti–*Teladorsagia circumcincta* immunoglobulin A.

around 6.24 kg compared with 6.65 kg. As with survival, there was poor support for a direct association between lactation and birth weight (fig. 5). The estimates for this direct effect were close to zero, and CIs overlapped substantially with zero (fig. 5). 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 (−0.0438 kg; 95% CI: −0.111.6 to −0.005.6); we examined the proportion of iterations that were lower than zero as a measure of significance and calculated that P_{MCMC} = .046.

In contrast to the other fitness-related traits investigated, there was 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; 95% CI: 5.9–11.2; figs. 3*D*, 5), 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 (95% CI: 0.12–1.75; figs. 3*D*, 4*D*, 5). 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 totaling 0.58 days' delay (95% CI: 0.06–1.31; fig. 5). Parturition date was thus the only metric examined here for which lactation's direct effect was larger than its indirect effect acting through strongyle count (figs. 3–5).

There was a strong positive association between total IgA and anti-Tc IgA, as expected given our previous findings (Albery et al. 2020*b*; table SI1; fig. SI1). However, lactation had no significant effect on anti-Tc IgA in our DAGs (figs. 3, SI1; table SI1).

Discussion

This study provides observational evidence for strong parasite-dependent mediation of multiple life-history trade-offs in a large wild mammal. Lactation was associated with higher parasite intensities, which translated to reduced
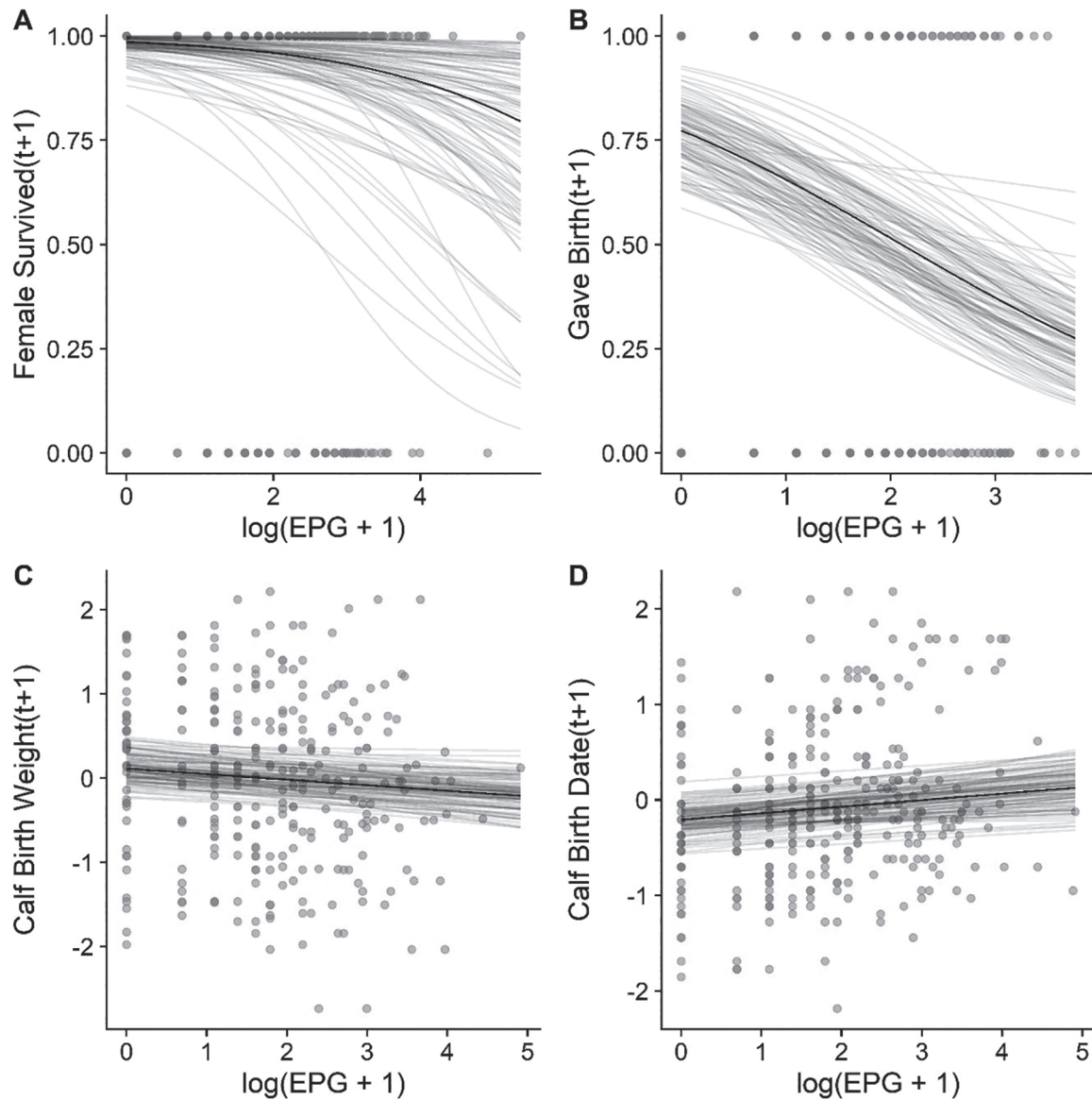
Figure 4: Relationships between strongyle parasite count and subsequent survival and fecundity in female red deer. Results are displayed for all four investigated fitness-related response variables: overwinter survival (*A*), fecundity (*B*), subsequent calf birth weight (*C*), and subsequent parturition date (*D*). The solid black lines denote the fitted slope of parasitism on the response variable. Transparent gray lines represent 100 draws from the posterior distribution to display variation in the estimated slope. Credibility intervals did not overlap with zero for any of the four relationships. Strongyle count was $\log(x + 1)$ transformed for analysis and plotting. EPG = eggs per gram.

fecundity and survival probability in the subsequent year. Among individuals that did reproduce the following year, those with high strongyle counts gave birth slightly later in the year and to slightly smaller calves. These findings represent the second evidence for such mediation of reproduction-survival trade-offs in a wild mammal (Leivesley et al. 2019) and new evidence of parasites mediating reproductive trade-offs with subsequent reproductive traits. It is likely that much of the subsequent reduction in survival

and reproductive fitness associated with lactation in the Isle of Rum red deer population (Clutton-Brock et al. 1989; Froy et al. 2016) is caused by strongyle parasites or that strongyle count closely corresponds to latent condition variables that are responsible for mediating fitness. This finding supports parasites' role as an important mediating factor in this system.

Lactation's negative association with subsequent fitness-related traits acted largely through strongyle count for all
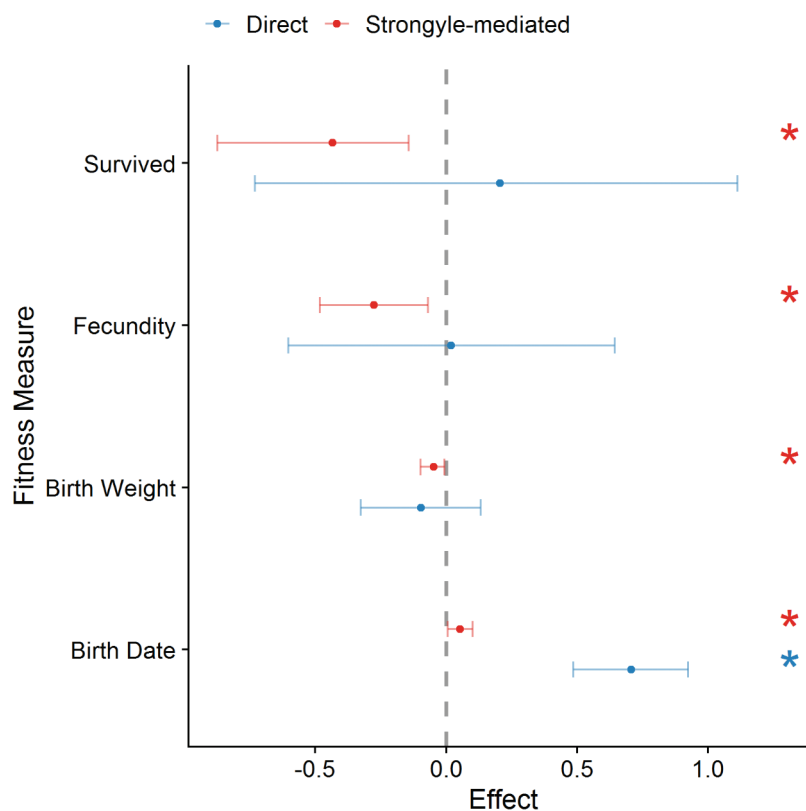
Figure 5: Comparison of direct and indirect (parasite-mediated) effects of lactation on fitness-related traits on the link scale (logistic for survival and reproduction, Gaussian for birth weight and birth date). Points represent mean effect estimates derived from the model posterior distributions; blue corresponds to direct effects, and red corresponds to indirect effects. Parameters with asterisks were significant at $P_{MCMC} < .05$.

response variables except parturition date. This may represent a parasite-mediated cost, where pathology and resource allocation associated with increased parasitism are the primary cause of increased overwinter mortality and reduced subsequent fecundity in lactating individuals (Clutton-Brock et al. 1989). Allocation of resources to lactation and associated physiological changes likely reduces resources available for resistance and damage repair mechanisms, rendering lactating females more susceptible to strongyles (Sheldon and Verhulst 1996; Speakman 2008). Notably, while most literature on reproduction-parasitism-fitness relationships revolves around energetic/resource trade-offs, it is also possible that nonenergetic mechanistic relationships between reproduction, physiology, and survival are partially responsible (Speakman 2008; Stahlschmidt et al. 2013); for example, the greater parasite counts in lactating individuals may be the product of greater exposure through heightened forage intake (necessitated by greater resource demand) rather than originating from increased susceptibility incurred by greater reproductive allocation (Albery et al. 2020b). Because strongyle intensity exhibits

strong seasonality, strongyle abundance on the pasture could likewise influence the shape of these trade-offs; however, because intensity peaks in spring (Albery et al. 2018), it is unlikely that variation in environmental abundance is responsible for driving the differential costs of gestation and lactation that we see.

High parasite counts in lactating females may cause gut pathology, interfering with nutrient absorption and thereby exacerbating the nutritional scarcity of the winter period, leading to overwinter 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). This reduction in body condition could likewise cause females to give birth later in the year and to a calf that is smaller. There are two time points at which strongyles may reduce fecundity. First, parasites may impact females in the resource-abundant summer and early autumn, preventing them from conceiving in the autumn mating season. In this case, strongyle-associated pathology may occur somewhat independently of overwinter nutritional scarcity.

Alternatively, strongyles may cause females to lose their pregnancies over winter. This possibility may be tested in the future by investigating whether more highly parasitized females are less likely to be observed mating (demonstrating reduced conception rates) or only less likely to give birth.

Lactation exerts a substantial resource cost that results in reduced condition; therefore, it is also possible that we observed a negative relationship between parasitism and fitness because both were determined by condition rather than because parasites were causally responsible for reducing fitness. Strongyle counts are associated with decreased body condition in shot individuals in this population, supporting this possibility (Irvine et al. 2006). Similarly, an important role for condition is supported by our observation that higher anti-Tc IgA levels were associated with increased fecundity the following year, independently of any associations between (1) strongyles and fecundity and (2) anti-Tc IgA and strongyles themselves (fig. 6). It is highly likely that anti-Tc IgA is well correlated with an unmeasured component of individual quality such as fat content (Demas et al. 2003), which is linked to fitness both in the deer (Albon et al. 1986) and in other systems (Milenkaya et al. 2015). This possibility reflects the con-
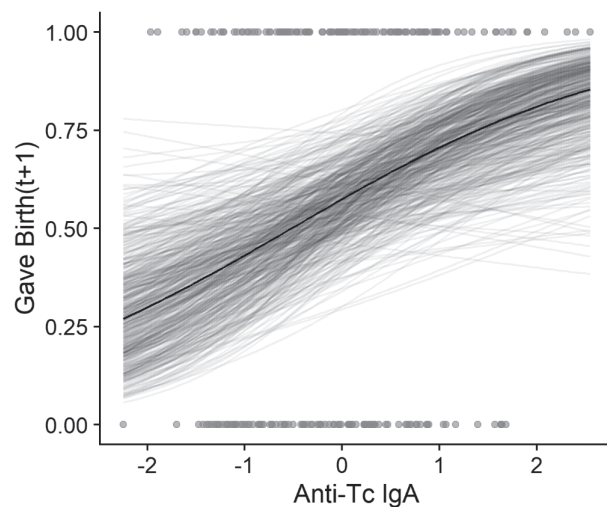

Figure 6: Higher anti–*Teladorsagia circumcincta* immunoglobulin A (anti-Tc IgA) was associated with an increased probability of reproducing the following year in female red deer. Anti-Tc IgA was cube root transformed and calculated from the residuals of a linear model including collection variables and was then scaled to have a mean of 0 and a standard deviation of 1. The lines represent the output of the reproduction probability model, which includes lactation and strongyles as explanatory factors. The solid black line represents the mean of the anti-Tc IgA effect in the reproduction probability model, which includes lactation and strongyles as explanatory factors. Transparent gray lines represent 500 draws from the posterior distribution to display variation in the estimated slope.

founding effects of individual quality in observational studies of trade-offs (van Noordwijk and de Jong 1986). We were unfortunately unable to replicate previous findings of lactation costs for mucosal antibodies (Albery et al. 2020*b*), likely as a result of extremely reduced sample sizes (485 samples and fewer here compared with 837 samples previously), and so we were unable to link the anti-Tc IgA–fitness association as part of a reproduction-fitness trade-off. Another potential fitness-mediating factor is body weight, which is often used to control for condition-driven versus parasite-driven fitness effects; however, analyses in Soay sheep often show that strongyle-fitness associations occur independently of or in addition to body weight (e.g., Sparks et al. 2018; Froy et al. 2019; Leivesley et al. 2019). In addition, although condition-parasitism relationships are well documented, negative effects are far from ubiquitous, and their slopes are relatively shallow on average (Sánchez et al. 2018). This difficulty untangling fitness consequences of parasitism from their condition-dependent correlates is indicative of a wider problem in observational disease ecology studies; specifically, confirming fitness consequences of infection often requires treatment with antiparasitics to compare the fitness of treated versus untreated individuals (Graham et al. 2011; Fenton et al. 2014; Pedersen and Fenton 2015). We emphasize that while path analysis is useful for inference of likely causal links (Shipley 2009), the approach 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, such as genetic variation. Path analysis must therefore be used carefully and in conjunction with specific a priori hypotheses and feasible causal pathways (e.g., see fig. 1).

Whether or not strongyles are the effectors, our findings nevertheless support the use of these parasites as a proxy for an individual's health and as predictors of its subsequent fitness. If our results are indicative of underlying fitness costs, strongyles will have a strong mediating effect on population dynamics for two principal reasons: first, by reducing both survival and fecundity simultaneously; and second, by exhibiting different relationships with past and subsequent reproduction. As such, it stands to reason that their impact will prevent females from reproducing in a single year, potentially stabilizing population fluctuations. Further years of data will reveal how parasite abundances relate to the population dynamics of the deer, and particularly whether interannual variation in strongyle numbers can explain population size (Wilson et al. 2004). At higher population densities the deer exhibit delayed maturity and reduced fecundity (Albon et al. 1983); the lactation-strongyle-fecundity trade-off offers a potential mechanism behind this fecundity reduction, particularly as parasitism should increase at higher densities

(Altizer et al. 2003; Wilson et al. 2004). Local population density also influences fitness-related traits in this population (Coulson et al. 1997) and parasitism demonstrates fine-scale spatial variation (Albery et al. 2019), so this life-history mediation could likewise occur at relatively fine spatial scales. A similar study in Soay sheep on Saint Kilda demonstrated that strongyles mediate a reproduction-survival trade-off but without examining similar reproduction-fecundity trade-offs, partly because most sheep do not take years off between reproduction events (Leivesley et al. 2019). The fecundity reduction seen in the deer and the strength of these parasite-mediated trade-offs potentially contribute to the population's relatively weak population fluctuations, particularly compared with the large fluctuations seen in the Soay sheep (Clutton-Brock and Pemberton 2004). As such, parasite-dependent life-history mediation may be an important contributing factor determining the strength of oscillatory population dynamics.

Finally, having uncovered costs of parasitism in adult females, it would be interesting to investigate whether other age and sex categories experience similar fitness effects: for example, do more highly parasitized males sire fewer calves, and are more highly parasitized calves less likely to survive to maturity? Do maternal costs transfer to their calves, providing another potentially important mediating mechanism (Martin and Festa-Bianchet 2010)? 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, quantifying transgenerational immunity-parasitism-fitness correlations—a topic that is largely understudied and likely influences ecological and epidemiological dynamics considerably (Roth et al. 2018).

Statement of Authorship

G.F.A. designed the study, collected samples, conducted laboratory work, analyzed the data, and wrote the manuscript. A.M. and S.M. collected samples and life-history data. F.K., D.H.N., and J.M.P. offered comments and suggestions on study design, theory, analysis, and the manuscript throughout.

Data and Code Availability

Data supporting this work are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.n8pk0p2t7; Albery et al. 2020a).

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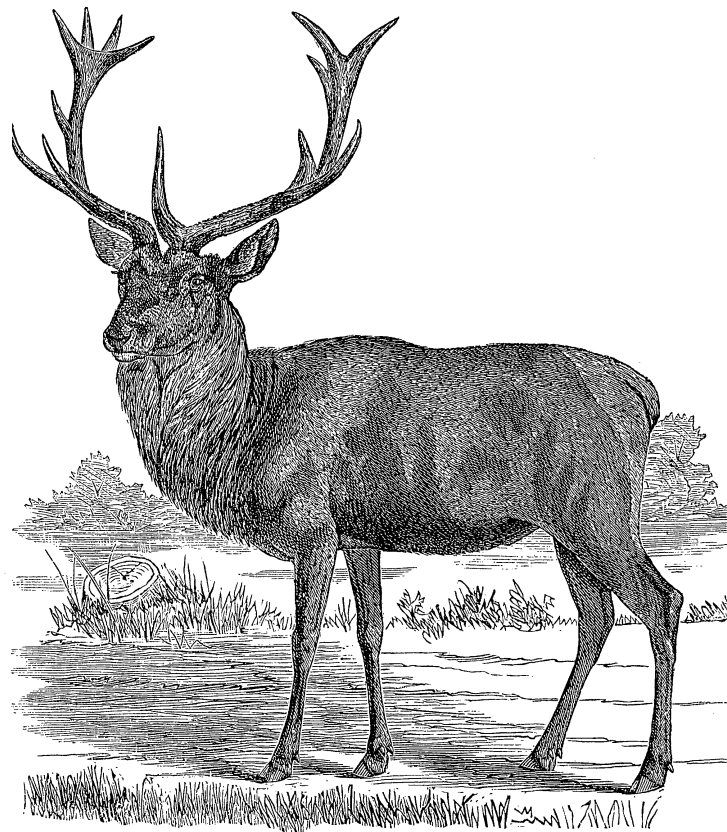
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