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Natural Selection on Antihelminth Antibodies in a Wild Mammal Population

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abstract: An effective immune response is expected to confer fitness benefits through improved resistance to parasites but also incur energetic costs that negatively impact fitness-related traits, such as reproduction. The fitness costs and benefits of an immune response are likely to depend on host age, sex, and levels of parasite exposure. Few studies have examined the full extent to which patterns of natural selection on immune phenotypes vary across demographic groups and environments in the wild. Here, we assessed natural selection on plasma levels of three functionally distinct isotypes (IgA, IgE, and IgG) of antibodies against a prevalent nematode parasite measured in a wild Soay sheep population over 26 years. We found little support for environment-dependent selection or reproductive costs. However, antibody levels were negatively associated with parasite egg counts and positively associated with subsequent survival, albeit in a highly age- and isotype-dependent manner. Raised levels of antiparasite IgA best predicted reduced egg counts, but this did not predict survival in lambs. In adults increased antiparasite IgG predicted reduced egg counts, and in adult females IgG levels also positively predicted overwinter survival. Our results highlight the potential importance of age- and sex-dependent selection on immune phenotypes in nature and show that patterns of selection can vary even among functionally related immune markers.

Keywords: ecoimmunology, fitness, strongyle nematodes, *Teladorsagia circumcincta*, immunoglobulin, Soay sheep.

Introduction

Parasites have a major impact on host condition and fitness and thereby represent a strong selective force for individuals in natural populations (Schmid-Hempel 2011). The main defense against parasites is the host immune system (Murphy 2012), and consequently it is expected for there to be a strong directional selection in favor of robust and effective immune responses in natural populations (Nunn et al. 2000; Lindström et al. 2004; Scharsack et al. 2007). Directional selection on a phenotypic trait is predicted to erode genetic variation in that trait (Falconer and Mackay 1996), but considerable genetic variation underlying parasite burden, infection risk, and immune phenotypes has been observed in humans and laboratory studies and in the wild (Lazzaro and Little 2009; Maizels and Nussey 2013). A key explanation for the maintenance of genetic variation in immune responses in the face of selection lies in the fact that mounting an immune response can incur considerable energetic costs (Lochmiller and Deerenberg 2000). Although investing in immunity may promote survival in the face of parasite infection, it draws resources away from other key fitness functions, such as growth and reproduction (Sheldon and Verhulst 1996). Alternatively, mounting strong immune responses can lead to damage to host tissue and autoimmunity, which would impose a fitness cost (Graham et al. 2005; Viney et al. 2005). These processes could result in a pattern of stabilizing—rather than directional selection—on immune phenotypes (Seppälä 2015).

Both the costs of investing in immune responses and the pattern of selection on immune traits are expected to vary with environment, sex, and age (Seppälä 2015). Parasite exposure risk is expected to vary over space and time, and the cost-to-benefit balance of investing in immunity to that parasite will therefore also vary (Altizer et al. 2006). Similarly, heightened intrasexual competition and immunosuppressive effects of testosterone experienced by males (particularly in polygynous species) leads to the expectation of differences

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in the fitness cost and benefits of investing in immunity between the sexes (Zuk 1990). Both immune phenotype and the ability to mount an effective response to infection also vary profoundly with host age (Palacios et al. 2011; Simon et al. 2015). In particular, young immunologically naïve hosts tend to have the highest infection rates and parasite burdens, and the fitness costs and benefits of investing resources in immunity during early life may differ profoundly from those in adulthood (Jackson et al. 2014). Given the complexity of the vertebrate immune system, it is also important to consider that the pattern of natural selection may also vary considerably across different components of the immune response and depend critically on the immune assays chosen in a particular study (e.g., Gonzalez et al. 1999; Råberg and Stjernman 2003; Parejo and Silva 2009; Nussey et al. 2014; Watson et al. 2016). For instance, the magnitude of response to a nonspecific challenge—such as the swelling response to phytohemagglutinin challenge widely used in ecoimmunology—may be informative about selection on the host's general capacity to respond to immune challenge but may not necessarily reflect how selection operates on specific immune responses to ecologically relevant infectious agents (Demas et al. 2011; Graham et al. 2011).

In natural populations, variation in resource acquisition will influence the total availability of resources for investment in immunity and other life-history traits (Siva-Jothy and Thompson 2002; Seppälä and Jokela 2010). This may generate positive associations between immune traits and fitness-related traits because of differences in host condition and resource acquisition and obscure trade-offs between immunity and life-history traits (van Noordwijk and de Jong 1986; Kraaijeveld and Godfray 1997; Reznick et al. 2000). Despite this, studies in natural vertebrate populations do provide support for the hypothesis that increases in measures associated with immune responsiveness predict improved survival (Saino et al. 1997; Christe et al. 1998, 2001; Merino et al. 2000; Nussey et al. 2014; Watson et al. 2016) but reduced reproductive performance (Ilmonen et al. 2000; Råberg et al. 2000; Bonneaud et al. 2003; Uller et al. 2006; Marzal et al. 2007; Gasparini et al. 2009; Graham et al. 2010; Hayward et al. 2014). There is also emerging evidence that selection on immune measurements may be environment dependent (Svensson et al. 2001; Calsbeek et al. 2008; Graham et al. 2010) and that patterns of selection may vary among immune measures (Gonzalez et al. 1999; Parejo and Silva 2009; Nussey et al. 2014; Watson et al. 2016). For example, antibody responses of wild blue tits to diphtheria and tetanus antigens showed positive linear and curvilinear relationships, respectively, with overwinter survival (Råberg and Stjernman 2003). To date, few studies have comprehensively tested for sex-, environment-, and age-dependent associations between immune measurements and subsequent survival and fecundity, while correcting for host body weight, in wild vertebrate populations.

Here, we examine the relationships among three functionally distinct isotypes (IgA, IgE, and IgG) of antinematode antibodies and nematode parasite burden, host body weight, survival, and fecundity in the free-living St. Kilda Soay sheep population. The Soay sheep are infected with a number of parasitic helminths, including a variety of gastrointestinal strongyle nematodes, largely comprised of the species *Teladorsagia circumcincta*, *Trichostrongylus axei*, and *Trichostrongylus vitrinus* (Wilson et al. 2004; Craig et al. 2006). Strongyle nematode burden, in combination with harsh winter weather and low food availability, are a strong selective force on the sheep (Gulland 1992; Wilson et al. 2004; Craig et al. 2006; Hayward et al. 2011). An exhaustive examination of both directional and stabilizing selection on strongyle fecal egg count (FEC) found evidence of age and environment dependencies: high FEC predicted reduced survival but only in lambs when population size was low (Hayward et al. 2011). Immunological research on domestic sheep and laboratory mice demonstrates that antibodies—particularly those of the functionally distinct isotypes IgA, IgE, and IgG—play an important role in both the development and maintenance of immunity to these parasites (Stear et al. 2009; Harris and Gause 2011). IgG is the most common antibody isotype in blood and extracellular fluid and has the longest half-life of all antibody isotypes. IgG is effective at activating the complement system, neutralizing toxins, and acting as an opsonin (Murphy 2012). In mice, IgG is crucially linked to protective immunity to helminth infections (Appleton and McGregor 1987; Blackwell and Else 2001; Harris et al. 2006; McCoy et al. 2008), which may be mediated by complement (Esser-von Bieren et al. 2013). IgA is the predominant antibody isotype at mucosal secretions and is associated with neutralizing pathogens in the gut. IgE has the shortest half-life of all antibody isotypes and is typically found bound to mast cells below the skin and mucosa. IgE is the antibody isotype most frequently linked to defense against helminths and mediates allergic inflammation via mast cell degranulation and the release of cytotoxic molecules from basophils and eosinophils (Murphy 2012). In domestic lambs, reduced FEC are associated with increased parasite-specific IgA levels directed at worm growth and subsequent female fecundity (Stear et al. 1995; Strain et al. 2002) as well as the arrestment of development of L4 larvae (Stear et al. 2004). In addition, IgE-dependent mast cell degranulation is associated with reducing adult worm burden (Stear et al. 1995; Huntley et al. 2001). In previous cross-sectional studies using samples collected in 1 year or a few years in wild Soay sheep, we have assayed antibodies against antigens from larval stages of *T. circumcincta*. We detected highly isotype-specific relationships with body weight and FEC and positive associations between anti–*T. circumcincta* IgG antibody levels and subsequent survival (Nussey et al. 2014; Watson et al. 2016). A larger-scale study spanning 11 years found that anti–*T. circumcincta* pan-isotype antibody levels were associated with reduced breeding success in the subsequent year for both adult males and females of high body weight (Hayward et al. 2014). This suggests that variation in immune defenses in Soay sheep may be maintained by opposing selection on survival and fecundity and that selection may be sex and environment dependent.

In this study, we use a much larger 26-year anti–*T. circumcincta* antibody data set to comprehensively assess how selection via survival and fecundity operates on these immune markers, including tests for stabilizing, sex-, age-, and environment-dependent patterns of selection. We also tested the associations between antibody levels at the time of measurement and strongyle FEC (as a proxy for parasite burden) and body weight (as a proxy for body condition) and determined whether associations with survival and fecundity were statistically independent of these two measures. We predict that (1) there will be a negative association between antibody measures and strongyle FEC, indicative of their involvement in resistance to infection (Stear et al. 2009); (2) there will be a positive association between body weight and antibody levels, indicative of animals in better condition being able to mount stronger immune responses (Siva-Jothy and Thompson 2002; Seppälä and Jokela 2010); (3) selection on immune responses will be strongest in lambs and males because they have the highest parasite infection intensities and lower annual survival rates (Clutton-Brock and Pemberton 2004; Craig et al. 2008); and (4) selection will be stronger in years of high population density when infection risk is higher (Seppälä 2015).

Methods

Study Population

The Soay sheep is a primitive breed of domestic sheep that was isolated on the island of Soay in the remote St. Kilda archipelago several millennia ago and has been living under unmanaged conditions and evolving under natural selection since then (Clutton-Brock and Pemberton 2004). In 1932, 107 Soay sheep were moved to the larger island of Hirta after the evacuation of all human residents. Approximately one-third of the population of these sheep live in the Village Bay area of Hirta, and these individuals have been the subject of a long-term study since 1985 (Clutton-Brock and Pemberton 2004). In April of each year, around 95% of all lambs born in this area are caught and individually tagged. Each August, as many sheep as possible from the study population are recaptured using temporary traps (Clutton-Brock and Pemberton 2004). At capture, animals are weighed and blood and fecal samples are collected. Whole blood samples are collected into heparin tubes and centrifuged at 1,008 *g* for 10 min, and plasma are removed and stored at −20°C. The majority of sheep deaths occur over winter, and the population dynamics of the Soay sheep are characterized by periods of low but rising population sizes followed by high mortality crash winters in which more than half the population can die (Clutton-Brock and Pemberton 2004). Regular censuses and mortality searches during the winter months result in the majority of carcasses being discovered, providing accurate death date information.

Breeding success for study individuals is known with high precision but is calculated differently depending on sex. For female annual breeding success, we used the number of offspring born to the female in the subsequent spring (derived from observational data), while for males we calculated the number of offspring sired by the ram and born in the subsequent spring (derived using genetic pedigree construction). The pedigree was constructed using 315 unlinked single-nucleotide polymorphisms (linkage disequilibrium $r^2 < 0.05$) with a minor allele frequency >0.4 using the R library sequoia (Huisman 2017). Total Village Bay population (referred to as population density) was used as a measure of environmental conditions because it captures annual variation in both food availability and nematode parasite exposure (Wilson et al. 2004; Hayward et al. 2014).

In this study, we included all animals that were caught and had plasma samples taken in August between 1990 and 2015. At the same time point, fecal samples are collected for the estimation of strongyle FEC. FEC is estimated from fecal samples as the number of eggs per gram using a modified McMaster technique (for full details, see Gulland and Fox 1992; Hayward et al. 2014).

Measurement of Antibodies

Antibody measurements were based on previously established protocols in this study population (Nussey et al. 2014). IgA, IgG, and IgE activity against antigens of the third larval stage of *Teladorsagia circumcincta* were measured using direct (IgA, IgG) and indirect (IgE) enzyme-linked immunosorbent assays (ELISAs; henceforth, anti-Tc antibodies). We used *T. circumcincta* L3 somatic antigen (Moredun Research Institute) as the capture antigen for all assays diluted to 2 μg/mL in 0.06 M carbonate buffer at pH 9.6. We added 50 μL of the diluted capture antigen to each well of a Nunc-Immuno 96-microwell plate, which was covered and incubated at 4°C overnight. After the wells were washed three times in Tris-buffered saline-Tween (TBST) using a plate washer, 50 μL of the Soay sheep plasma sample diluted 1∶50 for IgA and IgE and 1∶12,800 for IgG were added to each well. The plates were then covered and incubated at 37°C for 1 h. Plates were then washed five times with TBST, and 50 μL per well of rabbit anti-sheep IgA detection antibody conjugated to horseradish peroxidase (HRP; AbD Serotec AHP949P) diluted 1∶16,000 was added to the anti–*T. circumcincta* IgA assay, and 50 μL per well of rabbit anti-sheep IgG detection antibody conjugated to HRP (AbD Serotec 5184-2104) diluted 1 : 16,000 was added to the anti–*T. circumcincta* IgG assay. For the anti–*T. circumcincta* IgE assay, 50 μL per well of anti-sheep IgE (mouse monoclonal IgG1, clone 2F1; Moredun Research Institute) diluted 1 : 100 was added, followed by a 1-h incubation at 37°C and five washes with TBST, and then 50 μL per well of goat anti-mouse IgG1-HRP detection antibody (AbD Serotec STAR132P) were added diluted 1 : 8,000 in TBST. All plates were then incubated at 37°C for 1 h. Plates were then washed five times with TBST, and 100 μL of SureBlue TMB 1-Component microwell peroxidase substrate was added per well and left to incubate for 5 min in the dark at 37°C. Reactions were stopped by adding 100 μL per well of 1 M hydrochloric acid, and optical densities (ODs) were read immediately at 450 nm using a Thermo Scientific GO Spectrophotometer.

All results were recorded as OD values. In order to minimize confounding of capture year and age effects with plate to plate variation, each plate included samples from 2 years paired at random with different age groups on each plate. All plates were run in duplicate, and duplicate sample ODs were removed if the coefficient of variation between duplicates was >0.2 or the absolute difference between the duplicates was >0.2 OD units. We also checked the correlation of ODs across duplicate plates and reran both plates if the correlation coefficient was <0.8. No duplicate plates failed this check, and the number of samples that failed quality control per assay was 13 for IgA (seven lambs and six adults), eight for IgE (six lambs and two adults), and 27 for IgG (five lambs and 22 adults).

To reduce error due to within-plate variation, we included two sample free wells per plate (50 μL TBST added instead of sheep plasma) as blanks and two wells of positive controls. Positive controls for the IgE assay were serum from ewes trickle infected with *T. circumcincta* and, for the IgA and IgG assay, plasma from normal healthy nonimmunized domestic sheep. For subsequent analyses, the mean optical density ratio of each sample was taken according to the formula

$$\mathrm{OD} = \frac{\text{sample OD} - \text{blank OD}}{\text{positive control OD} - \text{blank OD}},$$

where the numerator was set to zero if the blank OD was greater than the sample OD (in order to avoid negative values).

Statistical Analyses

The final data set used in the analyses encompassed 6,398 samples from 3,114 individuals whose age was known. Five samples from very late-born lambs caught in August that were within 50 days of birth were excluded from the data set because of the potential presence of maternal antibodies and differences in development stage to other lambs. The final data set is deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.9qr0401 (Sparks et al. 2018).

All analyses were conducted using linear and generalized linear mixed models in R v3.4.4 (R Development Core Team 2016). Because of the immaturity of the immune system at 4 months (Smith et al. 1985) and the large increase in antibody levels between 4 and 16 months, we ran three models per response variable for lambs (both sexes), adult females, and adult males. Adults were separated into females and males because of the differences in range and distribution of phenotypic traits, such as weight and FEC in adults, as well as differences in survival rates and breeding success (fig. A1; figs. A1–A4 are available online). We built models with August weight, August strongyle FEC, overwinter survival, and annual breeding success as response variables with anti–*T. circumcincta* IgA, IgE, and IgG levels as explanatory variables. Continuous explanatory variables were rescaled to mean 0 and 1 SD before inclusion in each model subset. We first fitted a model containing the fixed effects previously identified as important predictors of these variables. We then simplified the model by stepwise deletion, sequentially removing fixed effects with the lowest t values and determining statistical significance using likelihood ratio tests until a base model containing only significant ($P < .05$) fixed effects was left. All dropped terms were then retested against this base model using the same criteria. Population size and sex (where fitted) were kept in all models in order to test for interactions with antibody levels. To the minimal model containing only significant terms, we separately added linear and quadratic functions of anti-Tc IgA, IgE, and IgG and assessed them for significance using likelihood ratio tests. Next we tested whether there were sex- or density-dependent effects of antibody levels in each of the models by looking at interactions with sex and population density against the linear and, if significant, quadratic antibody terms.

August strongyle FEC was heavily right skewed and zero inflated. Since the accuracy of the method used to estimate FEC is limited to 100 eggs per gram (Gulland and Fox 1992), we binned August FEC into multiples of 100, with zeros counted as 0 and values >6,000 eggs grouped into a single final bin (fig. A1*A*, A1*B*). Associations between antibody measures and August strongyle FEC were modeled using generalized linear mixed models via the glmmADMB package v0.8.3.3 (Fournier et al. 2012; Skaug et al. 2016). To assess which distribution best fitted the data set, we tested Poisson distributions and two negative binomial distributions (NB2 parameterization where variance $= \mu(1 + \mu/k)$ and NB1 fit where variance $= \varphi\mu$) with and without zero inflation and compared the Akaike information criterion (AIC) between all six models. We found that the negative binomial NB2 distribution without zero inflation had the lowest AIC for all models of FEC: lambs, adult females, and adult males. We included fixed effects for sex (lamb model only), weight,

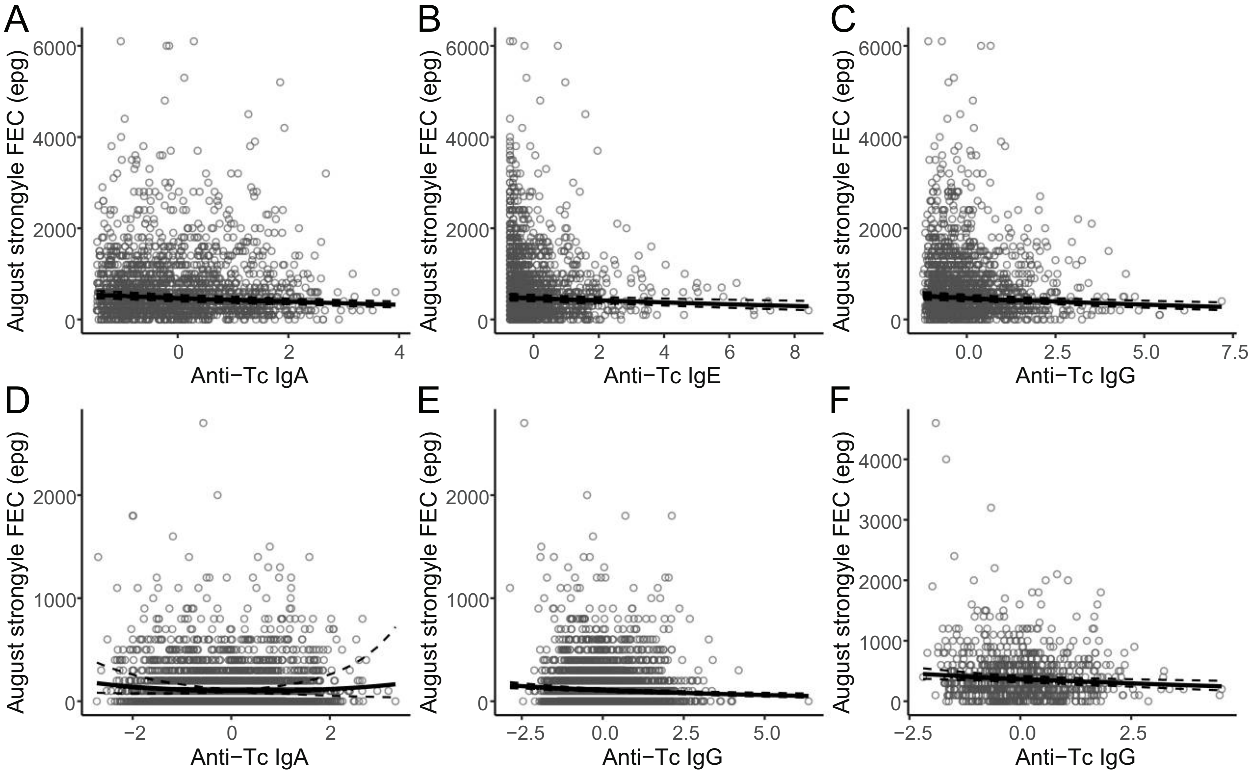

Figure 1: Scatterplots of raw data and generalized linear mixed model predictions for pairwise associations between anti–*Teladorsagia circumcincta* IgA, IgE, and IgG levels and August strongyle fecal egg count (FEC; eggs per gram [epg] with two outliers >6,000 eggs set as 6,100). Predictions are estimated at average values for all continuous fixed effects in the minimal model and singleton females for lambs and are rescaled to epg after (*A*–*C*), with dashed lines indicating 95% confidence intervals. Associations are shown between anti-Tc IgA, IgE, and IgG and strongyle FEC in lambs (*A*–*C*); anti-Tc IgA and IgG for adult females (*D*, *E*); and anti-Tc IgG for adult males (*F*).

age, and population density. Because previous studies of FEC in Soay sheep have identified curvilinear relationships between FEC and both age and weight (Hayward et al. 2009), we tested both linear and quadratic effects of these terms in our models. We included capture year as a random effect in both models and individual identity as a random effect in the adult models.

We investigated potential associations between August weight and anti-Tc antibody levels using linear mixed effects models via the lme4 package v1.1–17 (Bates et al. 2015), assuming a Gaussian distribution (fig. A1*C*, A1*D*). For the lamb models, we included sex, twin status, age in days, maternal age (quadratic), and population size as fixed effects and capture year and maternal identity as random effects. For adult weight models, we included age (linear and quadratic terms) and population size as fixed effects and individual identity and capture year as random effects.

We calculated overwinter survival as survival from capture in August to May 1 in the subsequent year using death date, capture, and census information. Survival probability varied with age and sex (fig. A1*E*) as well as among years, as previously documented (Clutton-Brock and Pemberton 2004). Analyses of survival were performed using generalized linear mixed models (GLMMs) in the lme4 package with a binomial error distribution and a logit link function. Binomial GLMMs were run with the built-in bobyqa optimizer, and the maximum number of iterations increased to $2E^{-6}$ to improve model convergence. For the lamb model, we included sex, twin status, weight (linear and quadratic), and population size as fixed effects and capture year as a random effect. For the adult models, we included age (linear and quadratic), weight (linear and quadratic), and population size as fixed effects, with individual identity and year as random effects.

For females that survived the winter, we calculated annual breeding success as the number of offspring born to a female in the subsequent spring from observational data. For male annual breeding success, we calculated the number of offspring sired by the ram and born in the subsequent spring using the pedigree. The male annual breeding success measure included males that died over the winter but excluded all males that were not seen to participate in the rut in the census records taken between October and December. The probability of producing a lamb was highly age and sex dependent (fig. A1*F*), with female and male lambs having particularly low probabilities of breeding (44% and 9%, respectively). Since the distributions of fecundity measures are very different for males and females (fig. A1*F*), sex-specific models were run for breeding success in both lambs and adults in this case. Female annual breeding success measures were treated as binary in both lambs and adults (lambed/did not lamb), while male fecundity measures were treated as binary only in lambs because of the low number of individuals siring lambs in their first year. All three models were run as a GLMM with a binomial error structure as for survival. For adult males, we analyzed annual breeding success in the glmmADMB package, where the best-fitting model was the negative binomial with the NB2 parameterization without zero inflation. For male and female lamb models, we included weight (linear and quadratic) and population size as fixed effects and year as a random effect. For adult male and female breeding success, we included age (linear and quadratic), weight (linear and quadratic), and population size as fixed effects and individual identity and year as random effects.

Results

The three anti-Tc antibody isotypes were positively but weakly to moderately associated with one another, with higher correlations among isotypes observed in lambs than in adults ($r = 0.28$–0.42 in lambs and 0.07–0.11 in adults; see fig. A2). We detail the associations found between antibody isotypes and strongyle FEC, weight, overwinter survival, and annual breeding success; a summary of these results can be found in figure A3.

Associations between Antibody Levels and Strongyle FEC

In lambs, August strongyle FEC was weakly but significantly negatively associated with all three anti-Tc antibody isotype (IgA, IgG, and IgE) levels (table 1; fig. 1*A*–1*C*). For all three isotypes, August FEC decreased linearly with increasing antibody levels. In a model containing all three antibody isotypes, IgA levels were independently negatively associated with FEC ($b = -0.074 \pm 0.021$ SE, $\chi^2_{(1)} = 11.860$, $P = .001$), while IgE and IgG were no longer significantly associated with FEC (IgE: $b = -0.030 \pm 0.020$ SE, $\chi^2_{(1)} = 2.180$, $P = .140$; IgG: $b = -0.034 \pm 0.022$ SE, $\chi^2_{(1)} = 2.420$, $P = .120$). We found no evidence for sex- or density-dependent effects of antibody levels on FEC in lambs (table 1).

In adults, there was a negative but weak linear association between anti-Tc IgG levels and August FEC for both males and females (table 1; fig. 1*E*, 1*F*). In females, there was also a weak curvilinear relationship of IgA with FEC (table 1; fig. 1*D*). A model with both significant antibody terms for females showed these two associations to be independent (IgG: $b = -0.116 \pm 0.029$ SE, $\chi^2_{(1)} = 15.780$, $P < .001$; IgA: $b(\mathrm{IgA}^2) = 0.312 \pm 0.120$ SE, $b(\mathrm{IgA}) = -0.339 \pm 0.119$ SE, $\chi^2_{(1)} = 6.720$, $P = .010$). Model predictions suggested that FEC was lowest at intermediate anti-Tc IgA levels in adult females (fig. 1*D*), and without the quadratic term in the model there was not a significant linear association between anti-Tc IgA and FEC ($b = -0.050 \pm 0.029$ SE, $\chi^2_{(1)} = 2.980$, $P = .084$). We found no evidence for density-dependent effects of antibody levels on FEC in adults (table 1).

Associations between Antibody Levels and Weight

There was a significant quadratic association between August weight and anti-Tc IgE levels in lambs (table 2). Model predictions suggested that weight increased and then plateaued with increasing IgE levels in lambs (fig. 2*A*). Without the quadratic in the model, there was a significant positive linear association between IgE and weight ($b = 0.128 \pm 0.042$ SE, $\chi^2_{(1)} = 9.399$, $P = .002$). This association was not driven by a few individuals with high anti-Tc IgE levels because a quadratic association remained after removing the 40 individuals with scaled IgE levels >3 ($b(\mathrm{IgE}^2) = -1.019 \pm 0.294$ SE, $b(\mathrm{IgE}) = 0.704 \pm 0.154$ SE, $\chi^2_{(1)} = 11.996$, $P = .001$). We found no association of the other antibody isotypes and weight in lambs or antibody × sex or density interactions (table 2).

In adult females, there was a positive but weak linear association between August weight and anti-Tc IgE levels, while a significant curvilinear association was found between IgA and IgG levels and weight (table 2; fig. 2*B*–2*D*). While higher IgE levels were associated with heaviest August weights, it was the intermediate anti-Tc IgA and IgG levels that were associated with heaviest weights (fig. 2*B*–2*D*). These associations were not driven by a few individuals with high anti-Tc antibody levels because the association remained after removal of individuals with high antibody levels of each isotype (after removal of IgA levels >2.5 [$n = 15$]: $\chi^2_{(1)} = 10.235$, $P = .001$; after removal of IgE levels >2.5 [$n = 61$]: $\chi^2_{(1)} = 6.981$, $P = .008$; after removal of IgG levels >3.75 [$n = 5$]: $\chi^2_{(1)} = 26.621$, $P < .001$). Linear associations between weight and IgA and IgG were weaker and less significant than quadratic effects (IgA: $b = 0.102 \pm 0.052$ SE, $\chi^2_{(1)} = 3.873$, $P = .049$; IgG: $b = 0.085 \pm 0.044$ SE, $\chi^2_{(1)} = 3.628$, $P = .057$). When all the significant antibody terms were in the same model, all remained significant, suggesting that each antibody isotype had independent associations with August weight (IgA: $b(\mathrm{IgA}^2) = -0.621 \pm 0.187$ SE, $b(\mathrm{IgA}) = 0.667 \pm 0.189$ SE, $\chi^2_{(1)} = 11.075$, $P = .001$; IgE: $b = 0.156 \pm 0.053$ SE, $\chi^2_{(1)} = 8.934$, $P = .003$; IgG: $b(\mathrm{IgG}^2) = -0.605 \pm 0.145$ SE, $b(\mathrm{IgG}) = 0.674 \pm 0.154$ SE, $\chi^2_{(1)} = 17.413$, $P < .001$). A marginal density-dependent association with IgA was found, indicating a slightly stronger increase with weight at high sheep densities (table 2). The

Table 1: Generalized linear mixed model results of the final minimal model for August strongyle fecal egg count (FEC) for lambs (both sexes), adult females, and adult males

					Adults							
	Lambs (both sexes)				Females				Males			
Fixed effects	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*
Intercept	1.556	.069			.096	.052			1.309	.069		
Sex (male)	.423	.039	113.220	<.001								
Twin	.183	.049	14.240	<.001								
Age					−.633	.111	. . .	. . .	.579	.172	. . .	. . .
Age^2					.600	.102	34.440	<.001	−.411	.139	8.740	.003
Weight	−.912	.127	. . .	. . .	−.399	.039	101.340	<.001	−1.055	.219	. . .	. . .
Weight^2	.700	.130	30.080	<.001					.698	.228	9.320	.002
Population size	.234	.064	10.980	.001	−.039	.048	.640	.424	.054	.064	.700	.403
Dropped:												
Weight^2							.900	.343				
Added:												
IgA	−.096	.019	25.460	<.001	−.348	.120	. . .	. . .			.460	.498
IgA^2			.660	.417	.309	.121	6.560	.010			.060	.807
IgE	−.059	.019	9.340	.002			.200	.655			1.220	.269
IgE^2			1.160	.282			.840	.359			.200	.655
IgG	−.075	.019	15.220	<.001	−.120	.029	16.880	<.001	−.089	.032	7.760	.005
IgG^2			2.140	.144			.060	.807			.360	.549
Sex × IgA			.080	.777								
Sex × IgE			.200	.655								
Sex × IgG			.020	.888								
Population × IgA			2.800	.094			1.760	.185			3.600	.058
Population × IgE			1.020	.313			.040	.842			2.840	.092
Population × IgG			.040	.842			3.520	.061			.580	.446
Population × IgA^2							.420	.517				

Note: Included are the estimated effects (estimate), standard errors (SEs), and significance of fixed effects based on a likelihood ratio test (LRT; *P*) where df = 1. Dropped fixed effects show the significance of adding dropped terms back into the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and the quadratic terms combined, and interactions were tested in addition to both terms. Lambs: $n = 1{,}850$ individuals; adult females: $n = 2{,}694$ observations, $n = 875$ individuals; adult males: $n = 845$ observations, $n = 451$ individuals.

interaction remained significant when the other antibody terms were included in the model ($b = 0.077 \pm 0.031$ SE, $\chi^2_{(1)} = 6.262, P = .012$). In adult males we found a positive but weak linear association of IgA with weight but no association with the other antibody isotypes or density interactions (table 2; fig. 2*E*).

Associations between Antibody Levels and Overwinter Survival

We found little evidence for associations between any of the three anti-Tc antibody levels and survival in lambs or adult males (table 3). Although there were no significant interactions between antibody levels and sex for lambs, we found a marginally significant interaction between anti-Tc IgA level and population density. In adult females, we found that anti-Tc IgG and IgE levels were significant predictors of overwinter survival (table 3; fig. 3). Females with higher IgG and IgE levels were more likely to survive the winter, independent of age and weight (table 3; fig. 3). In a model with both IgG and IgE fitted, IgE became nonsignificant, but IgG remained significant (IgE: $b = 0.149 \pm 0.083$ SE, $\chi^2_{(1)} = 3.301$, $P = .069$; IgG: $b = 0.217 \pm 0.084$ SE, $\chi^2_{(1)} = 7.151$, $P = .007$). In addition, IgG remained significant if strongyle FEC was included in the model ($b = 0.278 \pm 0.095$ SE, $\chi^2_{(1)} = 9.544$, $P = .002$) and if the one individual with unusually high IgG (>5) was dropped ($b = 0.235 \pm 0.085$ SE, $\chi^2_{(1)} = 8.240$, $P = .004$). There was no evidence for environment-dependent selection on adult females (table 3).

Associations between Antibody Levels and Annual Breeding Success

In females, we found a negative association between anti-Tc IgE levels as lambs and the probability of females breeding in their first year (table 4). Although our initial model detected a significant quadratic association between IgE and female first year breeding probability (table 4), visual inspection of

Table 2: Linear mixed model results of the final minimal model for August weight for lambs (both sexes), adult females, and adult males

					Adults							
	Lambs (both sexes)				Females				Males			
Fixed effects	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*
Intercept	12.869	.221			21.290	.130			27.194	.232		
Sex (males)	1.448	.079	306.950	<.001								
Twin	−3.299	.112	711.190	<.001								
Age (days)	.383	.051	54.670	<.001								
Age (years)					5.840	.107	. . .	. . .	14.191	.328	. . .	. . .
Age (years)^2					−4.337	.106	1259.800	<.001	−9.361	.316	555.040	<.001
Maternal age	4.236	.166	. . .	. . .								
Maternal age^2	−3.936	.166	482.220	<.001								
Population size	−.409	.201	4.153	.042	−.549	.110	19.051	<.001	−.444	.187	5.543	.019
Added:												
IgA			.306	.580	.753	.189	. . .	. . .	.361	.133	7.275	.007
IgA^2			.161	.688	−.671	.188	12.820	<.001			2.765	.096
IgE	.281	.086	. . .	. . .	.185	.052	12.654	<.001			1.744	.187
IgE^2	−.169	.083	4.191	.041			.003	.957			.001	.971
IgG			.531	.466	.754	.153	. . .	. . .			.292	.589
IgG^2			3.155	.076	−.660	.145	20.796	<.001			1.905	.168
Sex × IgA			.036	.851								
Sex × IgE			.794	.373								
Sex × IgG			1.788	.181								
Sex × IgE^2			.441	.507								
Population × IgA			.023	.881	.077	.031	6.196	.013			.192	.662
Population × IgE			.152	.697			.571	.450			3.062	.080
Population × IgG			1.100	.294			.785	.376			.088	.766
Population × IgA^2							.448	.503				
Population × IgE^2			1.402	.237								
Population × IgG^2							.708	.400				

Note: Included are the estimated effects (estimate), standard errors (SEs), and significance of fixed effects based on a likelihood ratio test (LRT; *P*) where df = 1. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and the quadratic terms combined, and interactions were tested in addition to both terms. Lambs: $n = 1{,}992$ individuals; adult females: $n = 2{,}975$ observations, $n = 918$ individuals; adult males: $n = 927$ observations, $n = 480$ individuals.

this relationship and the model predictions suggested that it could be driven by a handful of lambs with very high antibody levels. When the four individuals with high anti-Tc IgE values were dropped, the quadratic was no longer significant (after removal of IgE levels >4: $\chi^2_{(1)} = 0.906$, $P = .341$), but there was a significant negative linear relationship between IgE levels and first-year breeding probability ($b = -0.294 \pm 0.118$ SE, $\chi^2_{(1)} = 6.465$, $P = .011$; fig. A4*A*). We found no associations with any other antibody isotype or density interactions in female lambs. In males, we found a positive association between IgE levels as lambs and breeding success in their first year (table 4). Male lambs with higher IgE antibody levels were more likely to sire offspring in their first year (fig. A4*B*), and this association remained after dropping the six individuals with high IgE levels ($b = 0.353 \pm 0.113$ SE, $\chi^2_{(1)} = 9.037$, $P = .003$). We found no associations with any other antibody isotype or environment-dependent interactions in lambs.

In adult females, we found a curvilinear association of both anti-Tc IgA and anti-Tc IgG on breeding probability, but these associations were extremely weak and marginally significant (table 4). There was no underlying linear association between either isotypes and annual breeding probability (IgA: $b = 0.030 \pm 0.153$ SE, $\chi^2_{(1)} = 0.039$, $P = .843$; IgG: $b = -0.015 \pm 0.132$ SE, $\chi^2_{(1)} = 0.013$, $P = .908$). There was a weak and marginally significant positive association between IgG levels and the breeding success of adult males the following spring (table 4). We found no significant associations between annual breeding success and any other antibody isotypes or density interactions in adults.

Discussion

In this study, we have provided an unusually comprehensive assessment of how circulating antibody levels against a prevalent nematode parasite relate to parasite egg counts, host

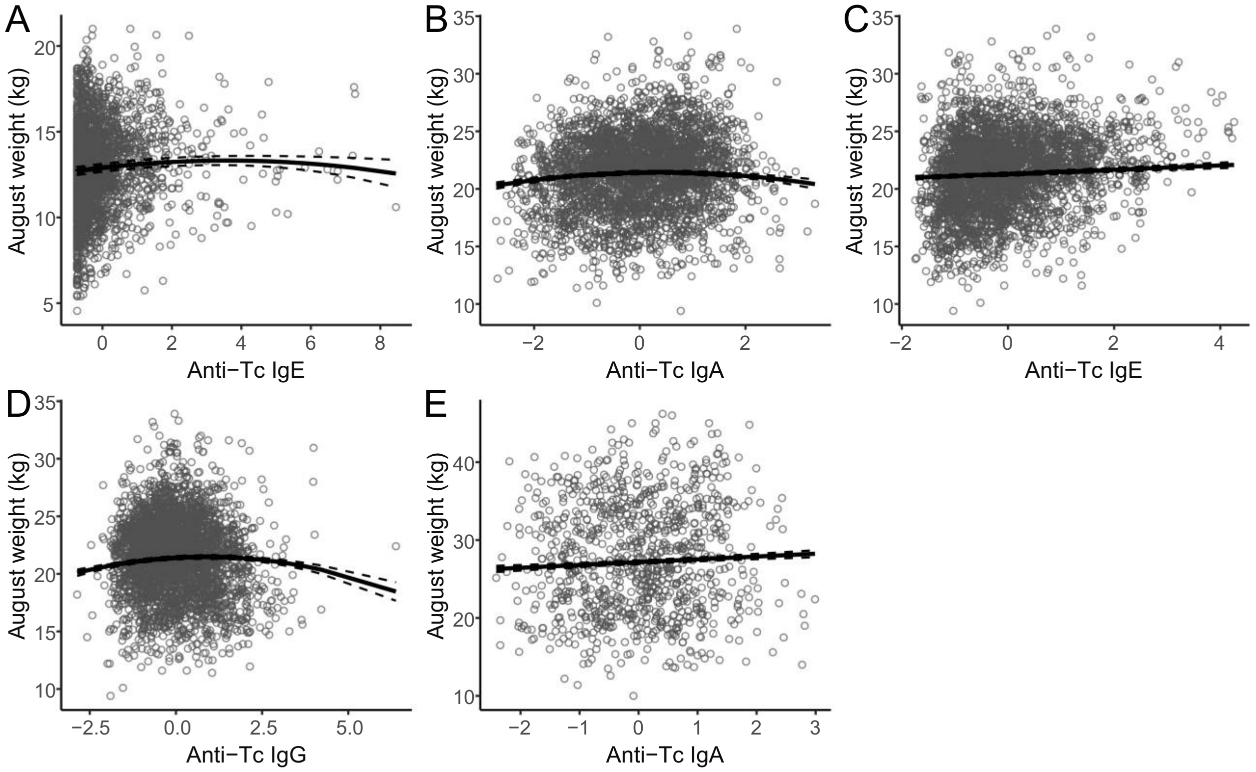

Figure 2: Scatterplots of raw data and linear mixed model predictions for pairwise associations between anti–*Teladorsagia circumcincta* IgA, IgE, and IgG levels and August weight. Predictions are estimated at average values for all continuous fixed effects in the minimal model and singleton females for lambs (*A*), with dashed lines indicating standard errors. Associations are shown between anti-Tc IgE and weight in lambs (*A*); anti-Tc IgA, IgE, and IgG and weight for adult females (*B*–*D*); and anti-Tc IgA for adult males (*E*).

body weight, and host fitness in a wild mammal population. Our data offer a rare opportunity to explore the relationships between markers of immunity against an ecologically important parasite group and fitness in both sexes under a highly variable natural environment. Unsurprisingly, given the considerable environmental heterogeneity in natural systems such as ours, the patterns of associations we observe are complex and vary even among closely functionally related antibody isotypes (for a summary of the results, see fig. A3). Our results provide important evidence for age-, sex- and isotype-dependent selection on antihelminth antibody levels, which may help explain how genetic variation in immune phenotypes is maintained in wild populations. Furthermore, several interesting and important patterns emerge from our analyses, most saliently (1) a clear age-dependent switch in the antibody isotype most strongly associated with FEC and fitness, which may offer important insights into how selection is operating on antihelminth immunity in natural vertebrate populations; (2) little consistent evidence for the expected consistent positive association between antibody measures and host body weight; and (3) limited support for environment-dependent selection, strong stabilizing selection, or costs associated with reproduction with any of the antibody measures we examined. We discuss each of these results and their implications in more detail.

Age-Dependent Associations among Antibodies, Strongyle Egg Counts, and Fitness

Generally, we found significant negative associations between anti-Tc antibody levels and strongyle FEC and positive associations with survival and reproduction, which is consistent with levels of these antibodies reflecting, in part, host resistance to nematode parasites rather than simply variation in parasite exposure (Hayward et al. 2014). However, the associations between antibody levels and strongyle FEC were typically weak, highlighting that numerous other factors beyond antibodies influence strongyle FEC, some of which have previously been identified in this study population (Wilson et al. 2004; Craig et al. 2008; Hayward et al. 2009, 2014). We also found evidence of a striking shift in the relationships between different antibody isotypes, worm egg counts, and overwinter survival probabilities with host age. IgA antibodies were the best predictor of FEC in lambs, but none of the isotypes

Table 3: Generalized linear mixed model results of the final minimal model for overwinter survival for lambs (both sexes), adult females, and adult males

					Adults							
	Lambs (both sexes)				Females				Males			
Fixed effects	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*
Intercept	.133	.242			3.103	.278			1.558	.344		
Sex (male)	−1.033	.119	78.850	<.001								
Age (years)					−1.463	.151	261.080	<.001	−1.082	.214	39.792	<.001
Weight	.797	.066	166.440	<.001	1.415	.137	210.990	<.001	1.205	.192	51.924	<.001
Population size	−1.241	.230	20.141	<.001	−.764	.250	8.471	.004	−.929	.335	7.652	.006
Dropped:												
Twin			.920	.337								
Weight^2			.441	.506			3.793	.051			.496	.481
Age^2							.320	.571			.599	.439
Added:												
IgA	−.093	.057	3.208	.073			.386	.535			2.444	.118
IgA^2			.004	.952			.005	.942			.014	.906
IgE			.223	.637	.178	.082	4.893	.027			<.001	.996
IgE^2			.115	.734			.990	.320			.118	.731
IgG			.017	.895	.239	.084	8.743	.003			<.001	.990
IgG^2			<.001	.987			1.693	.193			.159	.690
Sex × IgA			1.625	.202								
Sex × IgE			.248	.618								
Sex × IgG			.242	.623								
Population × IgA	−.129	.066	3.862	.049			.013	.908			.137	.712
Population × IgE			.336	.562			.039	.843			1.073	.300
Population × IgG			.594	.441			1.790	.181			.148	.701

Note: Included are the estimated effects (estimate), standard errors (SEs), and significance of fixed effects based on a likelihood ratio test (LRT; *P* value) where df = 1. Dropped fixed effects show the significance of adding dropped terms back into the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, effects are stated for both the linear and the quadratic terms combined, and interactions were tested in addition to both terms. Lambs: $n = 2{,}224$ individuals; adult females: $n = 2{,}935$ observations, $n = 903$ individuals; adult males: $n = 856$ observations, $n = 441$ individuals.

predicted lamb overwinter survival, while in adults IgG antibodies were the only isotype to predict FEC and also were positively associated with female overwinter survival and weakly with male annual breeding success. This pattern may reflect an important shift in selection on the immune response to parasitic helminths from initial exposure and the development of an immune response during early life to the mature adult immune response, which is thought to be more geared toward tolerating infection, wound healing, and limiting damage (Allen and Maizels 2011). Our results suggest that while antiparasite IgA levels may represent the best marker of the development of immunity to these parasites during early life, IgG represents the best marker of an adult's long-term ability to cope with persistent exposure and infection with nematode parasites. To our knowledge, our results provide the first evidence of such an age-related change in selection on immunity from the wild and provide an important basis for further studies to explore age-dependent changes in the immune response and its consequences for health and fitness under natural conditions.

Although all three antibody isotypes were moderately positively correlated in lambs (fig. A2) and were all significantly negatively associated with FEC when fitted separately in models, only anti-Tc IgA levels remained significant when all three isotypes were included in the model. This suggests shared covariance between isotypes and FEC, with IgA providing the strongest independent association. Veterinary studies of domestic sheep have demonstrated an important role of the mucosal IgA and IgE responses in the development of immunity to *Teladorsagia circumcincta* in lambs (Stear et al. 1995; Murphy et al. 2010). The hypersensitivity response (allergic inflammation caused by IgE-mediated mast cell degranulation) associated with production of antiparasite IgE is generally thought to be involved in the expulsion of larval parasites (McNeilly et al. 2009), while mucosal antiparasite IgA, which is positively correlated with circulating levels of antiparasite IgA (Henderson and Stear 2006), is thought to be involved in reducing adult worm growth and fecundity and inhibiting the development of worm larvae (Stear et al. 1995, 2004; Strain et al. 2002). Negative associa-

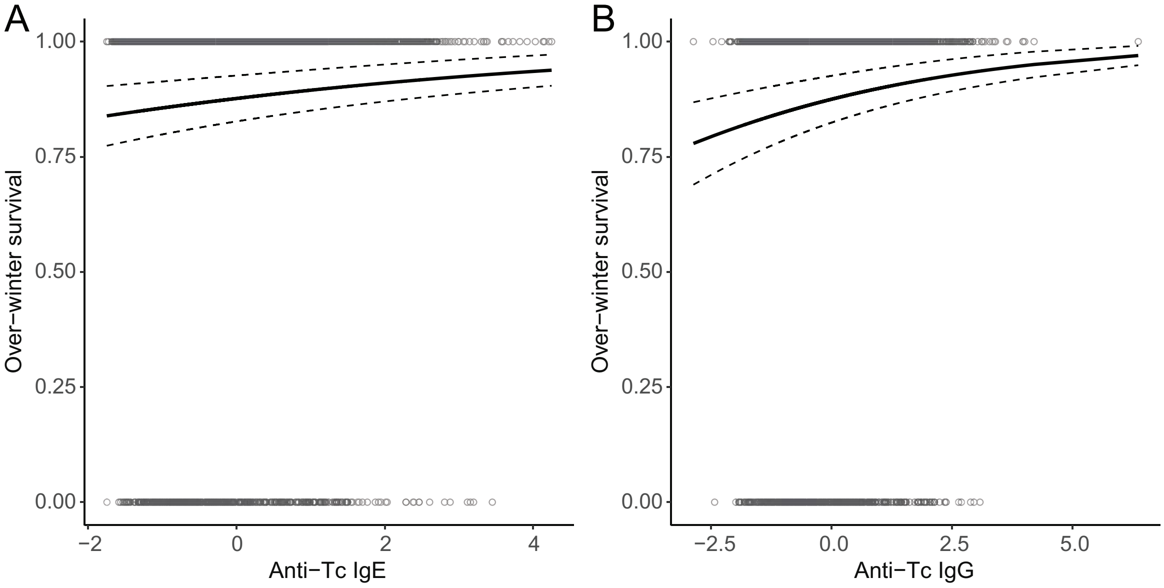

Figure 3: Scatterplots of raw data and generalized linear mixed model predictions for pairwise associations between anti–*Teladorsagia circumcincta* IgE (*A*) and IgG (*B*) levels and subsequent overwinter survival for adult female Soay sheep. The slope is predicted from the model with other fixed effects set as follows: data set average weight and age and a high Village Bay population size of 672.

tions between FEC and circulating anti-Tc IgA levels have been previously reported in both domestic and Soay sheep lambs (Stear et al. 1995, 2004; Coltman et al. 2001; Strain et al. 2002). Despite its negative association with FEC, we found little evidence that anti-Tc IgA levels in summer predicted first-winter survival or the probability of breeding the following year. This may be due to the immature, dynamic nature of the immune response at 4 months old, which may not reflect their anti-Tc antibody levels going into winter (Smith et al. 1985). Overall, the absence of relationships between anti-Tc antibody levels and first-winter survival in lambs was surprising, especially given that a previous study of Soay sheep found a negative association between strongyle FEC and annual fitness in lambs but not in adults (Hayward et al. 2011). This seems likely to reflect the greater importance of variation in exposure to helminths for health and fitness in immunologically immature lambs and contrasts with the patterns observed in immunologically mature adult sheep.

In adult Soay sheep there was a significant negative relationship between anti-Tc IgG and FEC in both sexes and significant positive associations between anti-Tc IgG and overwinter survival in females and annual breeding success in males. Anti-Tc IgA did independently predict FEC in adult females, but the relationship was not linear and implied slightly raised FEC at the extremes of the antibody distribution. Two previous smaller-scale, cross-sectional studies of this population investigating samples from different years have found that anti-Tc IgG levels predict overwinter survival in adult females, independent of other immune measures and body weight (Nussey et al. 2014; Watson et al. 2016). Observing the relationship again in our much larger-scale longitudinal study suggests that this association is observed only in adult females but is robust and independent of environmental conditions and individual body mass. Although studies on domestic ruminants have focused mainly on young animals (Stear et al. 2009), negative associations between anti-Tc IgG levels and FEC or worm number have been reported for adults (Williams et al. 2010; McBean et al. 2016), and there is a clear role for IgG in mediating protective immunity against helminths in laboratory mice (Blackwell and Else 2001; McCoy et al. 2008). It has been hypothesized that in early life, when animals are most vulnerable to helminth-associated morbidity, an aggressive immune response to limit worm establishment may develop, while in later life, once immune responses have matured and helminths have established infections, a more tolerant immune phenotype that limits worm fecundity, promotes healing of damaged tissues, and prevents immunopathology would be favored by natural selection (Allen and Maizels 2011; Medzhitov et al. 2012). Age-dependent selection on a tolerance-associated immune phenotype has been documented in wild voles, where increased GATA3 gene expression (a master regulator of the Th2 response) was positively associated with body condition and survival but only in older and larger males (Jackson et al. 2014). Our results provide support for age-dependent changes in the role of different antibody isotypes in resistance and tolerance to nematode parasites under natural infection. Further detailed immunological study is now required to understand

Table 4: Generalized linear mixed model results of the final minimal model for annual breeding probability of lambs and adults

	Females								Males							
	Lambs				Adults				Lambs				Adults			
Fixed effects	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*	Estimate	SE	LRT	*P*
Intercept	−.434	.199			6.468	.615			−3.100	.213			−.241	.123		
Age					1.857	.486	. . .	. . .					.203	.061	10.820	.001
Age^2					−2.349	.438	32.231	<.001								
Weight	.614	.109	35.592	<.001	2.148	1.059	. . .	. . .	4.540	1.560	. . .	. . .	.785	.076	102.700	<.001
Weight^2					−2.174	1.055	4.062	.044	−3.540	1.399	7.954	.005				
Population size	−.666	.194	9.553	.002	−.134	.125	1.069	.301	−.926	.145	26.737	<.001	−.240	.102	4.900	.027
Dropped:																
Age^2															1.820	.177
Weight^2			2.477	.116											.500	.480
Added:																
IgA			.766	.382	−1.176	.648	. . .	. . .			2.619	.106			1.760	.185
IgA^2			.410	.522	1.244	.649	4.075	.044			.149	.700			<.001	1.000
IgE	−.645	.230	. . .	. . .			.600	.439	.262	.090	7.634	.006			1.880	.170
IgE^2	.561	.254	6.183	.013			.916	.339			1.492	.222			.020	.888
IgG			.087	.768	−1.025	.557	. . .	. . .			.891	.345	.100	.050	4.040	.044
IgG^2			.481	.488	.991	.531	3.871	.049			.329	.566			.860	.354
Population × IgA			.089	.765			.981	.322			.118	.731			.220	.639
Population × IgE			.839	.360			.895	.344			.054	.816			1.760	.185
Population × IgG			.003	.955			.603	.437			.665	.415			3.400	.065
Population × IgA^2							1.130	.288								
Population × IgE^2			.023	.878												
Population × IgG^2							.787	.375								

Note: For lambs (both sexes) and adult females, the response is binary (lambed/did not lamb), while for adult males the response is a count of all lambs sired in the following spring. Included are the estimated effects (estimate), standard errors (SEs), and significance of fixed effects based on a likelihood ratio test (LRT; *P*) where df = 1. Dropped fixed effects show the significance of adding dropped terms back into the minimal model. For the added fixed effects, each antibody measure was added separately to the minimal model, including their linear and quadratic terms. Where the quadratic terms were significant, estimated effects are stated for both the linear and the quadratic terms combined, and interactions were tested in addition to both terms. Female lambs: $n = 592$ individuals; female adults: $n = 2{,}579$ observations, $n = 792$ individuals; male lambs: $n = 1{,}108$ individuals; male adults: $n = 887$ observations, $n = 457$ individuals.

why IgG antibodies provide the best marker of an individual's ability to cope with helminth infection in adulthood but not during early life.

Sex- and Environment-Dependent Selection on Antibody Levels

We found little evidence for sex differences in the associations between antibody levels and FEC or body weight, but marginal significant trends in our analysis suggest that relationships with survival and fecundity might differ between males and females. Perhaps most strikingly, in lambs there was a positive association between anti-Tc IgE and the probability of males producing offspring in the first year of life but a negative association in females (fig. A4). IgE antibodies are associated with a hypersensitivity response and mast cell degranulation in the gut, which is an effective mediator of nematode expulsion. It is also associated with inappetence, diarrhea, and reduced weight gain in domestic lambs (Stear et al. 2009). Given that males grow more rapidly than females in this species and body size is under strong sexual selection in male Soay sheep (Clutton-Brock and Pemberton 2004), it is surprising that an immune measure potentially associated with a cost to weight gain should be associated with improved reproductive performance in males but not females. We also found evidence for a weak and marginally significant positive association between anti-Tc IgG levels and adult male annual breeding success, while this isotype predicted overwinter survival in adult females. In wild ruminants generally, males are thought to invest a large amount of energetic resources in the autumn rut at a clear cost to their overwinter survival prospects, while females are more conservative and balance investment between their developing fetus and their own maintenance over winter (Clutton-Brock and Pemberton 2004). If antibody levels in summer reflect an individual's ability to resist nematode infections and limit the damage they cause, this may reduce the cost of infection in autumn and winter, freeing up resources that adult males would be expected to invest in the rut, while females invest in their own maintenance over winter. Overall, these results may reflect important and previously unknown differences in the costs and benefits between the sexes of mounting an aggressive hypersensitivity response to infection during early life and mounting a more tolerance-oriented response in adulthood. These trends warrant further investigation in both wild and domestic ruminant systems, involving more regular seasonal monitoring of reproductive hormones, immunity, and worm burdens across seasons in both sexes.

Given the considerable annual variation in environmental conditions and nematode parasite exposure experienced by Soay sheep on St. Kilda that is associated with often dramatic changes in population size (Clutton-Brock and Pemberton 2004), the general lack of evidence for interactions between antibody measures and sheep density in our models was surprising. We would predict the benefits, in terms of parasite burden and fitness, of improved immune responses to nematodes to be most strongly felt in years of high sheep density, when animals are in the poorest conditions and exposure to nematode larvae is highest (Hayward et al. 2014). However, there was very limited evidence for such relationships. This lack of environment-dependent associations may reflect the fact that anti-Tc antibody levels in Soay sheep appear highly repeatable in adulthood (Hayward et al. 2014; A. M. Sparks, unpublished data). Although there has been a far greater theoretical and empirical focus on environment-dependent selection on immune phenotypes as a force maintaining genetic variation in the immune response in natural systems (Svensson et al. 2001; Calsbeek et al. 2008; Graham et al. 2010; Seppälä 2015), our analyses provide far stronger support for age-dependent rather than environment-dependent selection. This argues for more theoretical and empirical effort being directed at investigating the evolutionary and ecological causes and consequences of such age-dependent variation in selection on immunity in the future.

Reproductive Costs and Condition Dependence

Unlike a number of experimental studies in wild birds, we found no strong evidence for the predicted negative association between parasite-specific antibodies and subsequent reproductive success, indicative of a reproductive cost of immunity (Ilmonen et al. 2000; Bonneaud et al. 2003; Marzal et al. 2007; Gasparini et al. 2009). There was a negative association between IgE levels and the probability of female sheep breeding in their first year; however, this result was only marginally significant. A previous study in Soay sheep found that high levels of self-reactive antinuclear antibodies were associated with reduced breeding success in both adult male and female Soay sheep (Graham et al. 2010). Furthermore, previous work using an anti-Tc antibody ELISA that simultaneously captures all isotypes (pan-isotype) found that adult males with high antibody levels were less likely to sire offspring the following year, and females of high body weight and high antibody levels were also less likely to have a lamb the subsequent year (Hayward et al. 2014). This study also found a marginal association between anti-Tc antibodies and survival in female lambs only (Hayward et al. 2014). This pan-isotype assay is expected to largely capture variation in IgG, which is the predominant isotype in circulation. However, these results contrast with the results of the current and previous isotype-specific studies (Nussey et al. 2014; Watson et al. 2016), suggesting that it is actually providing a different immunological signal to IgG when measured alone. One interesting possibility is that the pan-isotype measure better captures overall protein costs associated with antiparasite immune responses in general because it captures total immunoglobulin levels directed at these parasites. On the other hand, specific isotypes offer a clearer signal of specific immune responses directed at resisting or tolerating worm infection and thus are more strongly associated with survival. This difference among the current and previous study further highlights the complex and variable patterns of natural selection that can be observed even on apparently functionally closely related immune measures within the same study population.

There is a strong expectation for immune responses to be tightly linked with the condition of the host, with individuals in a better nutritional state more able to invest resources in immunity and therefore to resist infection (van Noordwijk and de Jong 1986; Kraaijeveld and Godfray 1997; Reznick et al. 2000). Interestingly, we found evidence for both positive linear and curvilinear relationships between body weight, depending on the age or sex group, and antibody isotype. However, these associations were typically weak, suggesting that anti-Tc antibody levels are not consistently or strongly positively associated with body weight of the host. A number of other studies have shown that body mass and food intake are closely related to immune responses (Siva-Jothy and Thompson 2002; Seppälä and Jokela 2010). However, in a previous study of a smaller sample of adult female Soay sheep, we did not find associations between anti-Tc antibody levels and weight (Nussey et al. 2014). Similarly, a recent study found that protein markers expected to be associated with nutritional state, total protein, and albumin were only weakly associated with body mass (Garnier et al. 2017). While body weight is often used as a proxy for condition in field studies, both our results and previous studies suggest that the relationship between body weight and levels of proteins in the blood that we expect to be intimately linked to nutritional state and physiological condition may be variable and weak (Hayward et al. 2014; Garnier et al. 2017). While our results highlight the point that using a few catch-all measures of immunocompetence is very unlikely to capture the complexity of the immune response and selection acting on it in wild systems (Demas et al. 2011), the complex relationships observed with body weight emphasize the need for biomarkers of nutritional state and condition within ecology (Garnier et al. 2017). Our study shows that closely related immune traits may have very different associations with health and fitness measures and suggests that multivariate immune phenotypes must be measured in order to fully understand how genetic variation is maintained in these traits in the wild.

Acknowledgments

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