Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep

B. H. CRAIG*, L. J. TEMPEST, J. G. PILKINGTON *and* J. M. PEMBERTON

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland

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SUMMARY

For hundreds of years, the unmanaged Soay sheep population on St Kilda has survived despite enduring presumably deleterious co-infections of helminth, protozoan and arthropod parasites and intermittent periods of starvation. Important parasite taxa in young Soay sheep are strongyles (*Trichostrongylus axei*, *Trichostrongylus vitrinus* and *Teladorsagia circumcincta*), coccidia (11 *Eimeria* species) and keds (*Melophagus ovinus*) and in older animals, *Teladorsagia circumcincta*. In this research, associations between the intensity of different parasite taxa were investigated. Secondly, the intensities of different parasite taxa were tested for associations with variation in host weight, which is itself a determinant of over-winter survival in the host population. In lambs, the intensity of strongyle eggs was positively correlated with that of *Nematodirus* spp. eggs, while in yearlings and adults strongyle eggs and coccidia oocysts were positively correlated. In lambs and yearlings, of the parasite taxa tested, only strongyle eggs were significantly and negatively associated with host weight. However, in adult hosts, strongyles and coccidia were independently and negatively associated with host weight. These results are consistent with the idea that strongyles and coccidia are exerting independent selection on Soay sheep.

Key words: Soay sheep, St Kilda, co-infection, body weight, *Trichostrongylus* spp., *Teladorsagia* spp., *Eimeria* spp., *Melophagus ovinus*.

INTRODUCTION

In natural host populations, component parasite communities are composed of a diversity of metazoan and protozoan parasite species so that mixed infections in individual hosts are normal. Many investigations on the nature of co-infections in wild mammals have been described (e.g. Montgomery and Montgomery, 1990; Haukisalmi and Henttonen, 1993; Behnke *et al*. 2005) but despite the fact that interactions between co-infecting species are commonly discovered wherever studied (Christensen *et al*. 1987) and that different parasite taxa exert different demands on the host immune system, few studies have attempted to elucidate the nature and evolutionary consequences of complex co-infections in the wild (Cox, 2001). Given that extracellular and intracellular parasites provoke different (Th1 and Th2) and mutually inhibitory host immune responses (Graham, 2002) and that appreciable heritable variation has been detected in resistance to parasites in the face of strong selection for resistance (Wakelin and Blackwell, 1988; Bishop *et al*. 1996; Coltman *et al*. 2001*b*), it seems probable that the evolution of resistance to any particular co-infecting species is subject to host genetic constraints and immunological compromise.

The feral Soay sheep population of Hirta, St Kilda represents a naturally parasitized population infected with taxonomically diverse parasites (Wilson *et al*. 2004; Craig *et al*. 2006, 2007). Previous research, largely focussing on the nematode parasites, has shown that high nematode burdens contribute to host mortality in high host density years (Gulland, 1992) making them a likely agent for natural selection. However, despite the deleterious impacts of parasites the population harbours heritable variation for nematode resistance (Smith *et al*. 1999; Coltman *et al*. 2001*a*), genetic variation associated with nematode resistance at the MHC (Paterson *et al*. 1998) and in the region of the γ-interferon gene (Coltman *et al*. 2001*b*) and potential QTL for coccidia resistance (Beraldi *et al*. 2007). This maintained genetic variation in parasite resistance could be explained if balancing selection for both extracellular (helminths and arthropods) and intracellular (e.g. protozoan) parasites was in operation.

This study represents the first attempt to investigate the potential role of co-infection as a selective force within the Soay sheep population. First, we provide a basic description of associations between helminth, protozoa and arthropod species across hosts. Second, we investigate whether the intensities of different parasite taxa have independent

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* Corresponding author: Wildlife, Ecology and Management Group H, Veterinary Surveillance, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK. Tel: +44 (0) 1904 462583. Fax: +44 (0) 1904 462111. E-mail: b.craig@csl.gov.uk

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associations with host weight, which is itself a predictor of over-winter survival and reproductive success in the study population (Clutton-Brock *et al.* 1992, 1997; Illius *et al.* 1995; Forchhammer *et al.* 2001).

MATERIALS AND METHODS

Population dynamics and parasitism

The feral Soay sheep population of Village Bay, Hirta, St Kilda, has been monitored extensively since 1985 and a detailed description of the population and its surveillance has been given by Clutton-Brock and Pemberton (2004). A wealth of data on the parasite taxa of the sheep exists. The host population shows extreme size fluctuation and this phenomenon is associated with the epidemiology of the different parasite taxa (Wilson *et al.* 2004; Craig *et al.* 2006, 2007). The analyses presented in this paper use counts obtained each August from 1993 to 2005. Some key findings about the epidemiology of the main parasite taxa are summarized below with more detailed accounts given elsewhere (Wilson *et al.* 2004; Craig *et al.* 2006, 2007).

Strongyles and coccidia

Wilson *et al.* (2004) give a thorough account of the patterns of strongyle egg output according to host sex and age and across seasons and years in the population. Generally, the counts are highly aggregated. Lambs and males have higher intensities and in high host density years the count is negatively associated with host survival (Illius *et al.* 1995; Coltman *et al.* 1999). As strongyle eggs can be produced by several different species, we can only speculate on those likely to be contributing to egg count in summer. From post-mortem worm counts obtained in the winter months of 1999, 2002 (Craig *et al.* 2006) and 2005 (Craig, unpublished data) it seems likely that species of the genus *Trichostrongylus* are significant contributors to egg count in lambs and yearlings, while *Teladorsagia circumcincta* probably contributes more to the counts in adults. The epidemiology of the 11 species of *Eimeria* in the population across 3 representative years of varying host density is detailed by Craig *et al.* (2007). Generally, intensity of all species tends to decrease with host age and varies with host population density.

Parasitology

Annual parasitological sampling and weighing takes place each August at a catch-up of individually-marked sheep that live in Village Bay. At the time of sampling, lambs and yearlings were 4 and 16 months old, respectively, and adults were 28 months and older. Assessment of *M. ovinus* (ked) infestation was made by a 1-min search for live keds in the sheep underbelly (area of white wool in wild type sheep). Diagnosis of endoparasite species infection was by coprological examination. Helminth eggs and coccidia (comprising a mixture of 11 *Eimeria* spp.; Craig *et al.* 2007) were quantified per gram of faeces using a modification of the McMaster technique (M.A.F.F., 1971). Helminth egg counts distinguished strongyle type egg (encompassing *Teladorsagia* spp., *Trichostrongylus axei*, *Trichostrongylus vitrinus*, *Bunostomum trigonocephalum* and *Chabertia ovina*), *Nematodirus* spp. (encompassing *N. battus* and *N. filicollis*), *Capillaria longipes* and *Trichuris ovis* and gave presence or absence of *Moniezia expansa*.

Statistical analysis

Separate analyses were conducted on lambs ($n=778$; one observation per individual), yearlings ($n=417$; one observation per individual) and adults (2 years and older; $n=1332$ observations on 561 individuals). Separate analyses were conducted within each age class because previous research indicated substantial differences in the predominant helminth species present at different host ages (Craig *et al.* 2006; see Discussion) and because different non-parasite covariates are relevant for August weight in different age classes; for example birth date, weight and litter size are important determinants of August body weight in lambs but not in older age classes.

(*i*) *Prevalence and intensity*. For each age class, the prevalence (%) and mean count for each parasite taxa was calculated for the period 1993–2005 in order to illustrate the variation in prevalence and intensity of the different taxa.

(*ii*) *Testing for associations between parasite taxa*. Our aim was to provide a basic description of the data rather than a detailed study of the possible ecological interactions between species. In common with much parasitological data, the distributions of intensity estimates for most species were not normal, so we used a non-parametric approach. Associations in the ranked intensity between species within each host age class were tested using Spearman's rank correlation tests. To limit the chances of making type 2 errors, a sequential Bonferroni correction (Rice, 1989) was applied to all tests (combining those conducted on lambs, yearlings and adults) yielding a threshold value of $P=0{\cdot}0015$. To check whether positive correlations were driven by hosts harbouring neither of the parasite taxon pairs, the analyses were repeated after excluding cases where neither parasite was present.

(*iii*) *Testing for associations between parasite taxa and August body weight in lambs*. A general linear model

Table 1. Overall prevalence (%) and mean count (±95% C.I.) of helminth and coccidia eggs/oocysts detected per gram of faeces and of keds detected from the underbelly of the sheep across all hosts and years (1993–2005) by age

	Lambs $n=778$		Yearlings $n=417$		Adults $n=1332$	
	Prevalence (%)	Mean count (±95% C.I)	Prevalence (%)	Mean count (±95% C.I.)	Prevalence (%)	Mean count (±95% C.I.)
Strongyles	95	719±50	86	407±48	58	176±14
Coccidia	99	5788±678	96	1078±100	77	558±44
Keds	82	3·36±0·24	28	0·29±0·06	12	0·15±0·01
Nematodirus	32	86·82±11·52	0·5	0·48±0·66	0·2	0·30±0·46
Moniezia	15	NA	13	NA	6	NA
Trichuris	2	3·21±1·99	0·7	0·72±0·81	0·07	0·07±0·15
Capillaria	0·4	0·38±0·43	3	2·88±1·74	0·6	0·67±0·49

(GLM) of August body weight was used to test for associations between parasite intensities and August weight in lambs. Year of birth (as a factor), day of birth (as a continuous variable), birth weight (as a continuous variable), litter size (as a factor at two levels; whether or not the lamb was a twin), day of sampling (as a continuous variable), sex (as a factor), coat type (as a factor at 4 levels), strongyle egg count, *Nematodirus* spp. egg count, *Capillaria* egg count, *Trichuris* egg count, *Moniezia* presence or absence (as a factor), mixed *Eimeria* spp. (coccidia) oocyst count and *Melophagus ovinus* (ked) count were fitted as potential explanatory variables. All two-way interactions between potential explanatory variables were also tested for. Body weight has a normal error structure and the final model's error structure was confirmed by checking the plot of residuals against standard normal deviates (Crawley, 2003).

(*iv*) *Testing for associations between parasite taxa and August weight in yearlings.* A general linear model (GLM) of body weight was used to test for associations between parasite intensities and August weight in yearlings. Year of birth, sex, coat type, horn type (all fitted as factors) and the same measures of parasitism as for lambs (above) were fitted as potential explanatory variables. All two-way interactions between potential explanatory variables were also tested for. As for lambs, body weight in yearlings has a normal error structure and the model was checked for non-normality of errors by assessment of the appropriate diagnostic plots.

(*v*) *Testing for associations between parasite taxa and August weight in adults.* To account for repeated observations, a general linear mixed model (GLMM) was applied (i.e. host identity was fitted as a random effect explicitly to account for repeated observations) to test for associations between parasite intensities and August weight in animals aged 2 years and older. Year of birth (as a factor), age (as a continuous variable), age^2 (fitted as a quadratic to account for the fact that weight increases to a peak value before decreasing with host age), sex, coat type, horn type (fitted as factors), strongyle egg count, presence/absence of *Moniezia* (as a factor), coccidia oocyst count and ked count were fitted as explanatory variables (there were not enough adult hosts infected with *Nematodirus* spp., *Capillaria* or *Trichuris* to justify the inclusion of these taxa). All two-way interactions between potential explanatory variables were also tested for. Body weight has a normal error structure and the model was checked for non-normality of errors by assessment of the appropriate diagnostic plots.

All statistical analyses were conducted in S-plus® professional edition version 6.2.1 for Microsoft windows (Insightful Corp.).

RESULTS

Parasite taxa prevalence and intensity

The parasite taxa with the highest prevalence and mean intensity in all age classes were strongyle eggs and coccidia oocysts (Table 1). Strongyles, coccidia, keds, *Nematodirus* and *Moniezia* had highest prevalence in lambs and decreased with age in terms of both prevalence and intensity. *Trichuris* and *Capillaria* had negligible prevalence and intensity in all age classes (Table 1).

Associations between parasite taxa

Spearman's rank correlation tests were applied for all possible pairings of parasite taxa in each age class (Table 2). Only 3 associations were significant after Bonferroni correction. In lambs, there was a positive correlation between strongyle and *Nematodirus* eggs ($r_s=0{\cdot}25$, $n=778$, $P<0{\cdot}0001$). In yearlings there was a positive correlation between strongyle eggs and coccidia oocysts ($r_s=0{\cdot}18$, $n=417$, $P<0{\cdot}001$). This positive association between strongyle eggs and coccidia oocysts was also found in adult hosts ($r_s=0{\cdot}299$, $n=1332$, $P<0{\cdot}0001$). These 3 associations were not driven by the presence of hosts with

Table 2. Spearman's rank correlation tests (r_s (P-value)) of intensity between different parasite taxa in lambs, yearlings and adults (no correction for repeated measures)

(Dashes denote that there were insufficient data points to perform a correlation. Significant values after sequential Bonferroni are highlighted in bold.)

		Nematodirus	*Trichuris*	*Capillaria*	Coccidia	Keds
Lambs (n=778)	Strongyles	**0·25 (P<0·0001)**	0·01 (P=0·68)	−0·06 (P=0·05)	0·05 (P=0·12)	0·08 (P=0·01)
	Nematodirus		0·007 (P=0·98)	0·012 (P=0·72)	0·09 (P=0·01)	0·04 (P=0·26)
	Trichuris			−0·009 (P=0·79)	0·05 (P=0·11)	0·06 (P=0·06)
	Capillaria				0·02 (P=0·48)	−0·02 (P=0·41)
	Coccidia					−0·008 (P=0·80)
Yearlings (n=417)	Strongyles	0·07 (P=0·15)	0·06 (P=0·24)	−0·02 (P=0·63)	**0·18 (P=0·001)**	−0·06 (P=0·13)
	Nematodirus		−0·006 (P=0·90)	−0·011 (P=0·82)	0·095 (P=0·05)	−0·036 (P=0·46)
	Trichuris			−0·014 (P=0·77)	0·06 (P=0·22)	−0·036 (P=0·47)
	Capillaria				−0·013 (P=0·79)	−0·016 (P=0·75)
	Coccidia					−0·05 (P=0·30)
Adults (n=1332)	Strongyles	—	—	—	**0·299 (P<0·0001)**	0·015 (P=0·57)
	Coccidia	—	—	—		0·031 (P=0·26)

Table 3. Results of a GLM of August body weight for lambs

(Of the parasite taxa tested, only strongyle egg count had a significant and negative association with host weight. Rejected terms were coat type, *Moniezia*, Keds, Coccidia, *Nematodirus*, *Trichuris* and *Capillaria*. Sample size was 778, no two-way interactions were significant, the final model had a normal error structure and explained 64·12% of the variance.)

Terms	D.F.	F	P-value	Association
Birth year	11, 760	11·74	<0·0001	Varies between years
Sex	1, 760	119·15	<0·0001	Males weigh more
Birth weight	1, 760	478·35	<0·0001	Heavier born weigh more
Birth date	1, 760	38·40	<0·0001	Later born weigh less
Litter size	1, 760	32·35	<0·0001	Twins weigh less
Capture day	1, 760	45·10	<0·0001	Later caught weigh more
Strongyles	1, 760	37·70	<0·0001	Hosts with higher egg counts weigh less

neither parasite. When double negative hosts were removed, the correlation between strongyle and *Nematodirus* eggs in lambs survived (r_s=0·22, n=747, P<0·0001), as did the correlation between strongyle eggs and coccidia oocysts in yearlings (r_s=0·16, n=413, P<0·001) and between strongyle eggs and coccidia oocysts in adults (r_s=0·131, n=1151, P<0·0001).

Associations with August weight

Over 778 lamb observations and 417 yearling observations, of all the parasite taxa tested, only strongyles were significantly and negatively associated with host weight measured in August (P<0·0001; Tables 3 and 4 and Fig. 1). Over 1332 observations on 561 adults, of the parasite taxa tested, only strongyle egg counts and coccidia oocyst counts were negatively and independently associated with August weight (P<0·0001 and P=0·0019 respectively; Table 5 and Fig. 2). No two-way interactions were significant in any of the models. Diagnostic plots confirmed that the final models had normal error structure.

DISCUSSION

Until recently the Soay sheep host-parasite system had been considered to be a relatively simple one. However, we now know that the situation is complex

Table 4. Results of a GLM of August body weight for yearlings

(Of the parasite taxa tested, only strongyle count had a significant and negative association with host weight. Rejected terms were horn type, *Moniezia*, Keds, Coccidia, *Nematodirus*, *Trichuris* and *Capillaria*. Sample size was 417, no two-way interactions were significant, the final model had a normal error structure and explained 50·0% of the variance.)

Terms	D.F.	F	*P*-value	Association
Birth year	12, 398	4·37	<0·0001	Varies between years
Sex	1, 398	293·53	<0·0001	Males weigh more
Coat type	3, 398	2·86	0·037	Dark > light, wild type > self
Strongyles	1, 398	76·84	<0·0001	Hosts with higher egg counts weigh less

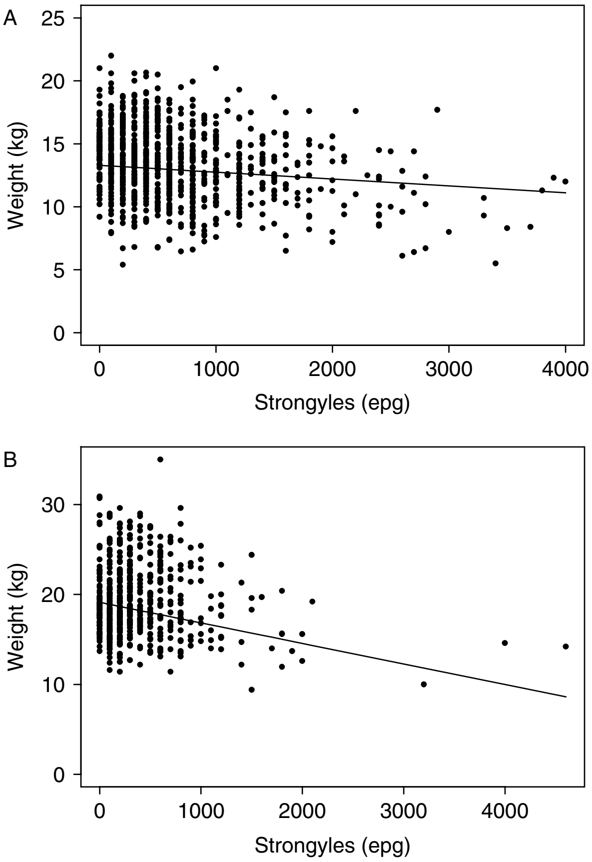

Fig. 1. Scatter plots showing negative association between host weight and strongyle egg count in (A) lambs ($n = 778$) and (B) yearlings ($n = 417$) measured in August using all available data from 1993–2005.

with no one nematode species achieving a monopoly throughout the lifetime of the host (Craig *et al.* 2006) and with a significant component of the parasite community comprised of a diverse array of protozoa (Craig *et al.* 2007). The potential role of metazoan-protozoan parasite co-infection as a selective force in the population has never been investigated. This study, therefore, attempted to initiate a debate on whether one parasite measure can capture the force of parasitism in the Soay sheep population simply because different species tend to be positively correlated or whether different taxa can exert independent effects on host weight and by inference, fitness.

Associations between metazoan and protozoan parasites

According to Lotz and Font (1991) an over-riding observation in studies of parasite infra-community structure and assembly is that generally the number of positive associations between species far outnumbers the negative associations. In this study there were several positive correlations of which 3 survived Bonferroni correction and re-analysis after dropping double negative cases. These comprise a correlation in ranked intensity between strongyle and *Nematodirus* eggs in lambs and a correlation between ranked strongyle eggs and coccidian oocysts in yearlings and adults. Although positive associations between parasite taxa may reflect synergistic effects of one taxon on another (Christensen *et al.* 1987) they may simply be a reflection of a similar response to host condition or co-variation in intake in parasites.

Associations between parasite taxa and host weight

The weight of Soay sheep in August has been shown to be important in virtually all analyses of their potential over-winter survival and reproductive success (Clutton-Brock *et al.* 1992, 1997; Illius *et al.* 1995; Forchhammer *et al.* 2001). In lambs and yearlings, of the various parasite taxa, only strongyle egg count explained variation in August weight. At this stage we can only speculate on the main nematode species contributing to egg count in young Soay sheep in August but from previous post- mortem studies performed on sheep in the winter months, high numbers of *Trichostrongylus axei* and *Trichostrongylus vitrinus*, together with a less numerous though emergent *Teladorsagia circumcincta* population were observed (Craig *et al.* 2006). In domestic sheep, *Trichostrongylus* spp. infection has been shown to be associated with host mortality (Gordon, 1950) and, furthermore, concurrent infections with *Trichostrongylus* and *Teladorsagia* species (as occurs in Soay lambs and yearlings) cause

Table 5. Results of a GLMM of August body weight in adults

(Of the parasite taxa tested, strongyle egg count and coccidian oocyst count had independent, negative associations with host weight. Rejected terms were coat type, horn type, *Moniezia*, Keds and *Nematodirus*. Sample size was 1332 observations on 561 individuals, no two-way interactions were significant, the final model had a normal error structure and explained 62·2% of the variance.)

Terms	D.F.	F	*P*-value	Association
Birth year	24, 535	4·55	<0·0001	Varies between years
Age	1, 767	193·83	<0·0001	Increases
Age^2	1, 767	155·06	<0·0001	Increases up to age 5–6, then declines
Sex	1, 535	1163·64	<0·0001	Males weigh more
Strongyles	1, 767	28·09	<0·0001	Hosts with higher egg counts weigh less
Coccidia	1, 767	9·69	0·0019	Hosts with higher oocyst counts weigh less

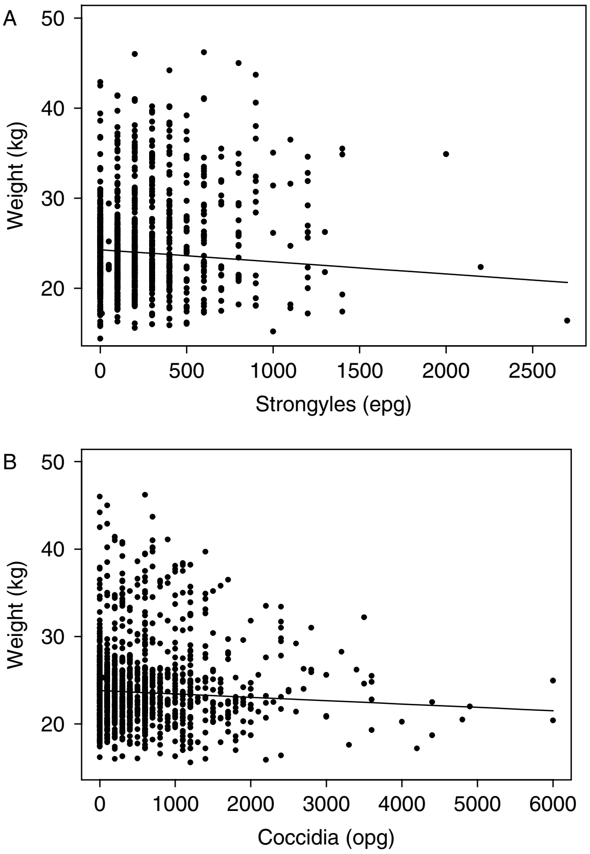

Fig. 2. Scatter plots showing negative associations between host weight and (A) strongyle egg count and (B) coccidia count in adults (2 years and older; $n=1332$) measured in August using all available data from 1993–2005.

even more severe pathology (Steel *et al*. 1982; Sykes *et al*. 1988). As sheep with higher August weight are more likely to survive over winter and strongyle egg count is negatively associated with over-winter survival in the population (Illius *et al*. 1995; Wilson *et al*. 2004) it seems possible that a synergistic effect of *Trichostrongylus* and *Teladorsagia* species infection could be exerting selection on Soay lambs and yearlings.

In adults it was found that both strongyle eggs and coccidian oocysts were independently negatively associated with host weight measured in August. Again, we can only speculate on the likely species involved. From winter post-mortem worm counts it is known that with increasing age, the once predominant *Trichostrongylus* genus becomes trivial in terms of intensity and *Teladorsagia circumcincta* monopolizes, therefore probably contributing almost all the eggs to the strongyle egg counts (Craig *et al*. 2006). In terms of species of *Eimeria*, an epidemiological study has shown that the only species to increase with host age in the sheep is *Eimeria granulosa* which was found to significantly co-occur with *E. weybridgensis* and *E. crandallis* (Craig *et al*. 2007). It can therefore be inferred that *Teladorsagia circumcincta*, parasitizing the abomasum, and *Eimeria* spp. (probably a combination of *E. granulosa*, *E. weybridgensis* and *E. crandallis*), parasitizing the small intestine, have the potential to exert selection on older Soay sheep independently of each other.

The observation that *Eimeria* spp. intensity is associated with depressed body weight in adult Soay sheep is of interest for two reasons. Firstly, we know of several studies that have demonstrated a deleterious effect of *Eimeria* spp. infection on host weight in domestic lambs (e.g. Chapman, 1974; Pout and Catchpole, 1974; Gauly *et. al*. 2004; Platzer *et al* 2005; Jorg Reeg *et al*. 2005) but the present study is the first we are aware of to suggest that there may be a detrimental effect on body weight in adult sheep.

Secondly, from an evolutionary perspective, metazoan-protozoan co-infections have the potential to exert complex demands on the host immune system which should evolve an optimal response (Graham, 2001). Regulated by the activity of cytokines, metazoan (extracellular) and protozoan (intracellular) parasites elicit a broadly dichotomous immune response (Graham, 2002). It is generally observed that helminth parasites provoke a Th2 response predominantly activated by the cytokine IL-4 which simultaneously inhibits Th1 responses.

Intracellular protozoa, such as *Eimeria*, are targeted by a Th1 response, activated by the cytokine interferon gamma, which in turn inhibits Th2 responses (Lydyard *et al*. 2001). As a consequence of the Th1 and Th2 dichotomy it is possible that in the Soay sheep on St Kilda there exists an immunological trade-off between resistance to metazoan and susceptibility to protozoan parasites which might explain why advantageous alleles conferring resistance to nematodes do not rise to fixation in the population as would be expected.

If a genetic trade-off does exist, we might have anticipated a negative phenotypic correlation between the strongyle and coccidia parasites instead of the positive correlation actually observed in yearlings and adults. There are several possible explanations for this observation. Apart from simply reflecting general host condition (van Noordwijk and de Jong, 1986) or parasite facilitation, there may also be more specific reasons for positive correlation between the two taxa. Firstly, there may be density-dependent effects in which intense infections of either *Teladorsagia circumcincta* or *Eimeria* spp. may cause fewer eggs or oocysts to be produced (Brackett and Bliznick, 1952; Williams, 1973; Bishop and Stear, 2000). Secondly, the mixed coccidia count, which is known to generally decrease with host age, may be masking an increase in intensity of an individual *Eimeria* species with a detrimental effect on host condition (see Craig *et al*. 2007). If this is the case then a balancing selection for resistance to the *Eimeria* spp. and *Teladorsagia circumcincta* could be in process (Craig *et al*. 2007).

Conclusion

Taken together, these results suggest a role for multiple parasite species in shaping the fitness and evolutionary trajectory of Soay sheep. In young Soay sheep, there is some evidence that the worm genus *Trichostrongylus* is a significant pathogen and that there may be synergistic effects of an emerging *Teladorsagia* infection with age, and these species are likely to be responsible for the detrimental association with body weight. In older sheep, strongyle eggs are likely to be produced predominantly by *Teladorsagia circumcincta*, and both this species and coccidia had independent negative associations with host weight. Since August weight is associated with the probability of over-winter survival, these results imply that at least 2 genera of parasite are likely to be exerting selection in the population, and for the first time suggest that intracellular (protozoa) as well as extracellular (strongyle) parasites are involved.

This work has, therefore, highlighted the need for further research in 2 main areas. Firstly there is a need to determine which nematode species are contributing to egg counts in the different age classes, not only in August, but throughout the year. Temporal variation in the abundance of the different parasite taxa is likely and strongyle and coccidia infections at other time-points in the year may be more important. Following this, there is a need to explore whether there are genetic trade-offs in resistance and susceptibility to infection with *Trichostrongylus* and *Teladorsagia* spp. and for infection with *Teladorsagia* and *Eimeria* spp. This would require further development of a molecular method developed by Wimmer *et al*. (2004) to provide quantitative species-specific data for strongyle eggs recovered from host faeces. If similar quantitative molecular methods could be developed to monitor protozoan species oocyst output, then this information, coupled with host pedigree information, could be used to estimate heritability for, and genetic correlations between, resistance to specific taxa, allowing hypotheses regarding associations with host genetic variation to be tested, including the possibility of balancing selection.

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