



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

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

Citation for published version:

Craig, BH, Tempest, LJ, Pilkington, JG & Pemberton, JM 2008, 'Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep', *Parasitology*, vol. 135, no. 4, pp. 433-441.
<https://doi.org/10.1017/S0031182008004137>

Digital Object Identifier (DOI):

[10.1017/S0031182008004137](https://doi.org/10.1017/S0031182008004137)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Parasitology

Publisher Rights Statement:

RoMEO green

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



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

(Received 16 July 2007; revised 9 November and 11 December 2007; accepted 11 December 2007; first published online 24 January 2008)

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

* 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

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 $P=0.0015$. 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 ($\pm 95\%$ 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 ($\pm 95\%$ C.I.)	Prevalence (%)	Mean count ($\pm 95\%$ C.I.)	Prevalence (%)	Mean count ($\pm 95\%$ C.I.)
Strongyles	95	719 \pm 50	86	407 \pm 48	58	176 \pm 14
Coccidia	99	5788 \pm 678	96	1078 \pm 100	77	558 \pm 44
Keds	82	3.36 \pm 0.24	28	0.29 \pm 0.06	12	0.15 \pm 0.01
<i>Nematodirus</i>	32	86.82 \pm 11.52	0.5	0.48 \pm 0.66	0.2	0.30 \pm 0.46
<i>Moniezia</i>	15	NA	13	NA	6	NA
<i>Trichuris</i>	2	3.21 \pm 1.99	0.7	0.72 \pm 0.81	0.07	0.07 \pm 0.15
<i>Capillaria</i>	0.4	0.38 \pm 0.43	3	2.88 \pm 1.74	0.6	0.67 \pm 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), age² (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 ($r_s=0.25$, $n=778$, $P<0.0001$). In yearlings there was a positive correlation between strongyle eggs and coccidia oocysts ($r_s=0.18$, $n=417$, $P<0.001$). This positive association between strongyle eggs and coccidia oocysts was also found in adult hosts ($r_s=0.299$, $n=1332$, $P<0.0001$). 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.)

		<i>Nematodirus</i>	<i>Trichuris</i>	<i>Capillaria</i>	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 ($P=0.01$)
	<i>Nematodirus</i>		0.007 ($P=0.98$)	0.012 ($P=0.72$)	0.09 ($P=0.01$)	0.04 ($P=0.26$)
	<i>Trichuris</i>			-0.009 ($P=0.79$)	0.05 ($P=0.11$)	0.06 ($P=0.06$)
	<i>Capillaria</i>				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 ($P=0.13$)
	<i>Nematodirus</i>		-0.006 ($P=0.90$)	-0.011 ($P=0.82$)	0.095 ($P=0.05$)	-0.036 ($P=0.46$)
	<i>Trichuris</i>			-0.014 ($P=0.77$)	0.06 ($P=0.22$)	-0.036 ($P=0.47$)
	<i>Capillaria</i>				-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

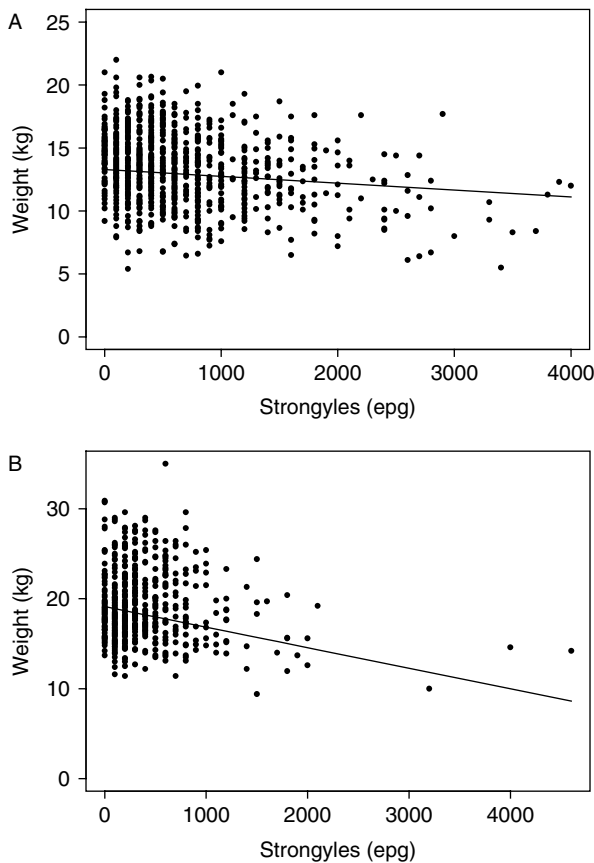


Fig. 1. Scatter plots showing negative association between host weight and strongyle egg count in (A) lambs ($n=778$) and (B) yearlings ($n=417$) measured in August using all available data from 1993–2005.

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 outnumber 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

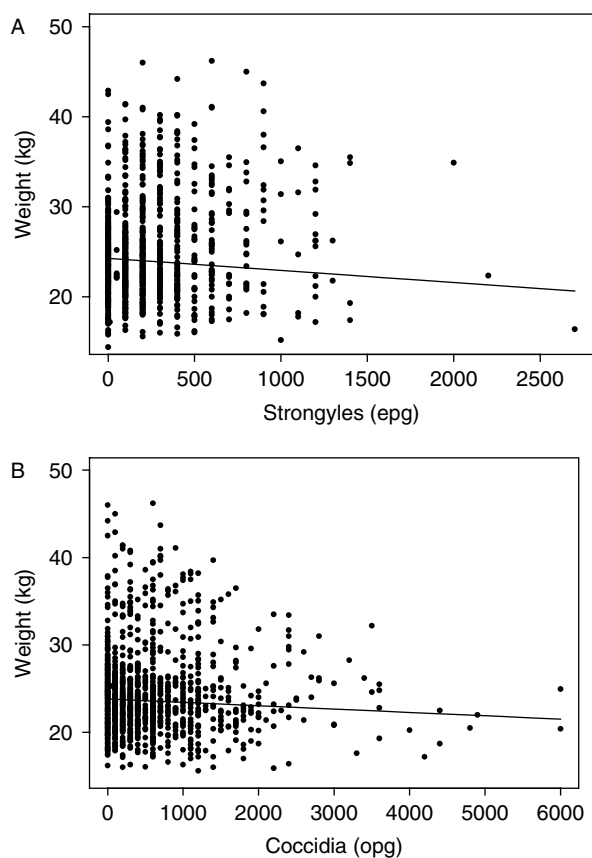


Fig. 2. Scatter plots showing negative associations between host weight and (A) strongyle egg count and (B) coccidia count in adults (2 years and older; $n=1332$) measured in August using all available data from 1993–2005.

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. weybridgeensis* 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. weybridgeensis* 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; Gaulty *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.

We thank The National Trust for Scotland and Scottish Natural Heritage for permission to work on St Kilda. Logistical support was kindly given by the MOD, QinetiQ and Eurest staff on St Kilda and Benbecula. Professors T. H. Clutton-Brock, B. T. Grenfell, M. J. Crawley and the Natural Environment Research Council are acknowledged for the long-term management and funding of the Soay sheep project Dr F. M. D. Gulland and many assistants and volunteers helped with collection of the field data. We thank Drs A. L. Graham, D. H. Nussey and K. Wilson for help, encouragement and suggestions for this study. Three anonymous referees also helped improve the manuscript. The project was funded by NERC and The Wellcome Trust.

REFERENCES

- Behnke, J. M., Gilbert, F. S., Abu-Madi, M. A. and Lewis, J. W.** (2005). Do the helminth parasites of wood mice interact? *Journal of Animal Ecology* **74**, 982–993.
- Beraldi, D., McRae, A. F., Gratten, J., Pilkington, J. G., Slate, J., Visscher, P. M. and Pemberton, J. M.** (2007). Quantitative trait loci (QTL) mapping of resistance to strongyles and coccidia in the free-living Soay sheep (*Ovis aries*). *International Journal for Parasitology* **37**, 121–129.
- Bishop, S. C., Bairden, K., McKeller, Q. A., Park, M. and Stear, M. J.** (1996). Genetic parameters for faecal egg count following mixed, natural, predominantly *Ostertagia circumcincta* infection and relationships with live weight in young lambs. *Animal Science* **63**, 423–428.
- Bishop, S. C. and Stear, M. J.** (2000). The use of a gamma-type function to assess the relationship between the number of adult *Teladorsagia circumcincta* and total egg output. *Parasitology* **121**, 435–440.
- Brackett, S. and Bliznick, A.** (1952). The reproductive potential of five species of coccidia of the chicken as demonstrated by oocyst production. *Journal of Parasitology* **38**, 133–139.

- Chapman, H. D.** (1974). The effects of natural and artificially acquired infections of coccidia in lambs. *Research in Veterinary Science* **16**, 1–6.
- Christensen, N. O., Nansen, P. and Fagbemi, B. O.** (1987). Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitology Research* **73**, 387–410.
- Clutton-Brock, T. H. and Pemberton, J. M.** (2004). *Soay Sheep: Dynamics and Selection in an Island Population* (ed. Clutton-Brock, T. H. and Pemberton, J. M.) Cambridge University Press, Cambridge.
- Clutton-Brock, T. H., Price, O. F., Albon, S. D. and Jewell, P. A.** (1992). Early development and population fluctuations in Soay sheep. *Journal of Animal Ecology* **61**, 381–396.
- Clutton-Brock, T. H., Wilson, K. and Stevenson, I. R.** (1997). Density-dependent selection on horn phenotype in Soay sheep. *Philosophical Transactions of the Royal Society, B* **352**, 839–850.
- Coltman, D. W., Pilkington, J. G., Smith, J. A. and Pemberton, J. M.** (1999). Parasite mediated selection against inbred Soay sheep in a free-living island population. *Evolution* **53**, 1259–1267.
- Coltman, D. W., Pilkington, J., Kruuk, L. E. B., Wilson, K. and Pemberton, J. M.** (2001 *a*). Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution* **55**, 2116–2125.
- Coltman, D. W., Wilson, K., Pilkington, J. G., Stear, M. J. and Pemberton, J. M.** (2001 *b*). A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. *Parasitology* **122**, 571–582.
- Cox, F. E. G.** (2001). Concomitant infections, parasites and immune responses. *Parasitology* **122** (Suppl.), S23–S38.
- Craig, B. H., Pilkington, J. G. and Pemberton, J. M.** (2006). Gastrointestinal nematode species burdens and host mortality in a feral sheep population. *Parasitology* **133**, 485–496.
- Craig, B. H., Pilkington, J. G., Kruuk, L. E. B. and Pemberton, J. M.** (2007). Epidemiology of parasitic protozoan infections in Soay sheep (*Ovis aries* L.) on St Kilda. *Parasitology* **134**, 9–21.
- Crawley, M. J.** (2003). Model criticism. In *Statistical Computing: An Introduction to Data Analysis Using S-Plus*. pp. 305–322. John Wiley & Sons Ltd, Chichester, UK.
- Forchhammer, M. C., Clutton-Brock, T. H., Lindstrom, J. and Albon, S. D.** (2001). Climate and population density induce long-term cohort variation in northern ungulate. *Journal of Animal Ecology* **70**, 721–729.
- Gauly, M., Reeg, J., Bauer, C. and Erhardt, G.** (2004). Influence of production systems in lambs on the *Eimeria* oocyst output and weight gain. *Small Ruminant Research* **55**, 159–167.
- Gordon, H. M.** (1950). Some aspects of parasitic gastro-enteritis of sheep. *Australian Veterinary Journal* **26**, 14–28.
- Graham, A. L.** (2001). Use of an optimality model to solve the immunological puzzle of concomitant infection. *Parasitology* **122** (Suppl.), S61–S64.
- Graham, A. L.** (2002). When T-helper cells don't help: immunopathology during concomitant infection. *The Quarterly Review of Biology* **77**, 409–434.
- Gulland, F. M. D.** (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology* **105**, 493–503.
- Haukisalmi, V. and Henttonen, H.** (1993). Coexistence in helminths of the bank vole *Clethrionomys glareolus*. 1. Patterns of co-occurrence. *Journal of Animal Ecology* **62**, 221–229.
- Illius, A. W., Albon, S. D., Pemberton, J. M., Gordon, I. J. and Clutton-Brock, T. H.** (1995). Selection for foraging efficiency during a population crash in Soay sheep. *Journal of Animal Ecology* **64**, 481–492.
- Jorg Reeg, K., Gauly, M., Bauer, C., Mertens, C., Erhardt, G. and Zahner, H.** (2005). Coccidial infections in housed lambs: oocyst excretion, antibody levels and genetic influences on the infection. *Veterinary Parasitology* **127**, 209–219.
- Lello, J., Boag, B., Fenton, A., Stevenson, I. R. and Hudson, P. J.** (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature, London* **428**, 840–844.
- Lotz, J. M. and Font, W. F.** (1991). The role of positive and negative interspecific associations in the organization of communities of intestinal helminths of bats. *Parasitology* **103**, 127–138.
- Lydyard, P. M., Whelan, A. and Fanger, M. W.** (2001). Cytokine families. In *Immunology* (ed. Hames, B. D.), pp. 87–91. Bios Scientific Publishers, Oxford, UK.
- M.A.F.F.** (1971). *Manual of Veterinary Parasitological Laboratory Techniques*. HMSO, London.
- Montgomery, S. S. J. and Montgomery, W. I.** (1990). Structure, stability and species interactions in helminth communities of wood mice *Apodemus sylvaticus*. *International Journal for Parasitology* **20**, 225–242.
- Paterson, S., Wilson, K. and Pemberton, J. M.** (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proceedings of the National Academy of Sciences, USA* **95**, 3714–3719.
- Platzer, B., Prosl, H., Cieslicki, M. and Joachim, A.** (2005). Epidemiology of *Eimeria* infections in an Austrian milking sheep flock and control with diclazuril. *Veterinary Parasitology* **129**, 1–9.
- Pout, D. D. and Catchpole, J.** (1974). Coccidiosis of lambs. V. The clinical response to long term infection with a mixture of different species of coccidia. *The British Veterinary Journal* **130**, 388–399.
- Rice, W. R.** (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
- Smith, J. A., Wilson, K., Pilkington, J. G. and Pemberton, J. M.** (1999). Heritable variation in resistance to gastro-intestinal nematodes in an unmanaged mammal population. *Proceedings of the Royal Society of London, B* **266**, 1283–1290.
- Steel, J. W., Jones, W. O. and Symons, L. E. A.** (1982). Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia*

- circumcincta*. *Australian Journal of Agricultural Research* **33**, 131–140.
- Sykes, A. R., Poppi, D. P. and Elliot, D. C.** (1988). Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus colubriformis* on the performance of growing lambs consuming fresh herbage. *Journal of Agricultural Science* **110**, 531–541.
- Van Noordwijk, A. J. and de Jong, G.** (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist* **128**, 137–142.
- Wakelin, D. and Blackwell, J.** (1988). *Genetics of Resistance to Bacterial and Parasitic Infection*. Taylor and Francis, London.
- Williams, R. B.** (1973). Effects of different infection rates on the oocyst production of *Eimeria acerulina* or *Eimeria tenella* in the chicken. *Parasitology* **67**, 279–288.
- Wilson, K., Grenfell, B. T., Pilkington, J. G., Boyd, H. E. G. and Gulland, F. M. D.** (2004). Parasites and their impact. In *Soay Sheep: Dynamics and Selection in an Island Population* (ed. Clutton-Brock, T. H. and Pemberton, J. M.) pp. 113–165. Cambridge University Press, Cambridge.
- Wimmer, B., Craig, B. H., Pilkington, J. G. and Pemberton, J. M.** (2004). Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. *International Journal for Parasitology* **34**, 625–631.