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Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep

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Livestock breeding programmes have created resistant (R) and susceptible (S) sheep that differ in their ability to control parasites through their immune function but potentially also their grazing behaviour (i.e. parasite avoidance). Using the Perendale genetic lines, we tested the hypothesis that R-sheep avoid parasites more effectively, reducing their parasite exposure/challenge, compared with S-sheep. However, in grazing systems, parasite-rich areas are also forage rich, suggesting that parasite avoidance behaviours are associated with nutritional penalties. We first created a naturally heterogeneous sward structure of gaps and tussocks and then used focal behavioural observations to quantify the sward selection of R- and S-sheep. Tussock swards were more nitrogen rich (41%), offered increased forage intake rates (32%) and contained 17 times more parasite larvae than gap swards. All the animals avoided grazing the tussock swards. However, the R-sheep grazed the tussock swards to a lesser degree than the S-sheep. We conclude that selection for genetic resistance has resulted in animals that, despite being well armed to fight parasitism through improved immune function, adopt parasite avoidance strategies with associated nutritional disadvantages. This experiment highlights the role of host behaviour in the control of parasitism and suggests that animals can be bred to avoid disease.

Keywords: parasite resistance; parasite avoidance; grazing behaviour

1. INTRODUCTION

Deposition of faeces by herbivores in pastoral environments results in localized concentrations of forage resources and parasites (Hutchings *et al.* 2002*a*,*b*). This heterogeneous distribution is created through the avoidance of faeces- and, thus, parasite-contaminated pasture by herbivores (e.g. sheep, Hutchings *et al.* 2001). The localized concentrations of forage resources (nutrients) and parasites are seen as relatively tall tussocks of grass. Grazing herbivores must then trade-off the nutritional benefits associated with faeces-contaminated tussocks against the increased risks (costs) of parasitism. The grazing decisions of herbivore hosts faced with this dilemma determine their intake of nutrients and parasites, and thus their fitness and survival in natural systems or productivity in agricultural systems. Livestock breeding programmes have created resistant and susceptible sheep that differ in their ability to combat parasites through their immune function (Donald 1994; Diez-Tascon *et al.* 2005). The grazing decisions of genetically resistant animals faced with this dilemma might be argued in two ways: do resistant animals select the nutritional advantages accepting the increased exposure to parasites as their improved immune function will combat the infection or do they accept the nutritional penalties associated with parasite avoidance thereby increasing their perceived resistance?

We address these questions using extreme, low (resistant R) and high (susceptible S) faecal egg counts (FECs; a measure of the concentration of parasite eggs in faeces, eggs per gram), Perendale selection lines that were initiated in 1984 in New Zealand. The Perendale lines had experienced 6 years of selection based on lamb FECs following experimental *Haemonchus contortus* parasite challenge (oral dosing of parasites under laboratory conditions), and thus the ability to combat gastrointestinal parasites via enhanced immune function. Microarray analysis has identified genes involved in acquired immune responses that are differentially expressed in the Perendale selection lines (Diez-Tascon *et al.* 2005). After the period of selection under experimental challenge, the Perendale lines experienced 13 years of selection (using the same criteria on each year's lambs) based on FECs during mixed gastrointestinal nematode parasite challenge through grazing (i.e. natural challenge), during which time selection could have continued being not only for immune function but also for selective grazing (i.e. parasite avoidance; see flock history below). Both immune function and avoidance of parasites by hosts influence parasite excretion levels and may have been included in the selection programme under natural challenge. The overall aim of our experiment was to determine whether R-sheep avoid suprapopulations of parasites in the environment more than S-sheep.

2. MATERIAL AND METHODS

The experiment was conducted at the AgResearch Woodlands Research Station, New Zealand where a 1 ha site was allocated for the experimental field plot. The plot was established on a mixed grass/clover pasture (perennial ryegrass, *Lolium perenne*; Kent Wild White, *Trifolium repens*). The experimental timetable (a total of 12 weeks) was divided

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into: (i) a 10-week period, during which the short inter-tussock/tall tussock sward structure was created and (ii) a subsequent 9-day behavioural observation period. Weekly faecal sampling to monitor the parasitic status of experimental animals started at the beginning of the behavioural observation period and continued for five weeks (i.e. sampling continued after the end of the behavioural observations). The experimental design was based on (i) a pool of 50 Perendale ewes (containing a natural mixed nematode infection) set stocked to create a inter-tussock/tussock sward mosaic subsequently grazed by (ii) 20 parasite-naive (raised under minimal parasite challenge through the use of clean pastures and anthelmintics) experimental female weaned lambs (approx. three months old): 10 R-sheep and 10 S-sheep balanced for live weight (R-sheep = Perendale resistant selection line (mean live weight ± s.e. = 22.4 ± 0.72 kg); S-sheep = Perendale susceptible selection line (mean live weight ± s.e. = 22.3 ± 0.58 kg) grazing the natural inter-tussock/tussock mosaic (inter-tussock = relatively short, non-contaminated swards; tussocks = relatively tall, faeces-contaminated swards). Lamb mean live weights (± s.e.) at the end of the experiment (five weeks after the start of the behavioural observations) were R-sheep = 28.8 ± 2.43 and S-sheep = 28.8 ± 1.47.

(a) *Flock history*

Both selection lines originated from the same Perendale flock. Perendale lambs Born in 1984 and 1985 were screened for extremely high and low FEC score. Each lamb was experimentally challenged with 250 infective *H. contortus* (L3) larvae per kilogram live weight after weaning. Infections were stopped with anthelmintic 63 days after initial infection and challenged again with 350 L3 larvae per kilogram live weight 14 days later. Selection of ram and ewe replacements was based solely on FEC ranking during peak egg excretion (five to seven weeks after infection). At least three sires were used in each line annually. This selection procedure was carried out until 1990 when the challenge regime was changed to a natural mixed infection (mainly *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*) during grazing (all else remained the same; Morris *et al.* 2005). The lines continue to be maintained under the same protocols to the present day. See Morris *et al.* (2005) for a full history of the lines and selection procedures.

(i) *Measurements*

Weekly faecal sampling started at the beginning of the behavioural observation period and continued for five weeks (i.e. sampling continued after the end of the behavioural observations) and were taken from the rectum of sheep and analysed for nematode eggs per gram of faeces (epg). Animal live weights, stratified sward heights, tussock availability and vegetation samples were taken at days 1, 5 and 10 (the day after behavioural observations ceased) of the behavioural observation period. Twenty sward surface heights were taken using a sward stick from both faeces-contaminated tussocks and non-contaminated inter-tussock swards while walking a *W*-transect. Tussock availability was determined by walking a *W*-transect in each plot and recording which vegetation type (inter-tussock/tussock) was present at the toe of each step (Hutchings *et al.* 2002*a*). Three composite mean sward samples of inter-tussock and tussock vegetation were collected on each sampling day by clipping small areas of sward (approx. 1 cm^2 and less than 0.5% of the tussock area) across the field plot and analysed for dry matter (DM) content, nitrogen (N) content and digestibility (organic matter digestibility; OMD). This method ensured minimal sampling impact on sward structure and availability during the behavioural observation period. At the end of the experiment, 10 tussocks were chosen at random in the experimental field plot. The tussocks were clipped using hand shears to the soil surface to create a total tussock sward sample. The inter-tussock vegetation surrounding the tussock was sampled by clipping herbage to the soil surface to create an inter-tussock sample. Sward nematode larval counts were then determined for each of the resultant 20 samples. Five hundred random pluck samples were taken from the remaining inter-tussock and tussock vegetation at the end of the experiment using thumb and forefinger to estimate the relative amount of herbage that would be consumed by the sheep when grazing the two sward types.

(ii) *Animal grazing behaviour and sward patch selection*

Direct observations were used to record both grazing behaviour and sward (inter-tussock/tussock) use of the sheep. On each observation day, an observer recorded the behaviour of individuals for 5 min during periods of activity. The order in which the animals were sampled was randomized. All individuals were recorded at least twice a day. A total of 362 double-blind 5 min recording periods over 9 days were carried out on the 20 sheep. The following grazing variables were recorded in each 5 min observation:

(i) number of bites taken from the inter-tussock and tussock patches. A bite was defined as a head pull associated with the severing of herbage.
(ii) bite rate (bites per second).
(iii) number of steps taken (steps per second). A step was defined as the forward and/or backward motion of a chosen front leg.

(iii) *Statistical analyses*

Analysis of variance was used to determine how sward type (i.e. inter-tussock/tussock) and day (days 1, 5 and 10) affected the DM content (g kg^{-1}), N content (g kg^{-1} DM) and *in vitro* OMD (g kg^{-1} DM) of swards. A generalized linear mixed model (GLMM) with a logit link and binomial error distribution was used to estimate the mean values of the proportion of bites taken from tussocks (tussock use). Residual maximum likelihood (REML; Patterson & Thompson 1971) was used to estimate the mean values for grazing parameters (bite rate (bites per second) and step rate (steps per second). The GENSTAT GLMM and REML procedures (Lawes Agricultural Trust 1993) included animal and day within animal (i.e. bottom stratum) as random effects, and day and selection line (i.e. resistant or susceptible) and their interaction as fixed effects. FEC and sward larval count (SLC; larvae kg^{-1} DM) were analysed using a generalized linear model (GLM) with a logit link and a negative binomial error structure (Wilson & Grenfell 1997). The experiment comprised two divergent lines, without replication. Formally, the selection lines would need replication to determine whether any differences in behaviour were indeed the consequences of the selection and not a consequence of genetic sampling/drift.

Table 1. Effects of sward type on sward characteristics. (Values are means of sward height, nitrogen content (N) and OMD measured in g kg^{-1} DM, and sward strongyle larval counts (SLC; larvae kg^{-1} DM). Composite mean DM weight (g) of 500 pluck samples is also given. Effects: n.s. $= p > 0.05$, $^{***}p < 0.001$.)

		sward		overall		effects		
		inter-tussock	tussock	mean	SED	day	sward	day × sward
height	day 1	2.82	9.51	6.17				
	day 5	3.23	11.03	7.13				
	day 10	3.72	13.04	8.38	0.333	***	***	***
N	day 1	35.0	50.6	42.8				
	day 5	30.5	44.0	37.3				
	day 10	25.9	42.7	34.3	2.89	***	***	n.s.
OMD	day 1	812	841	826				
	day 5	798	816	807				
	day 10	758	796	777	11.1	***	***	n.s.
SLC	day 10	524	6501	3512	2057	—	***	—
DM of 500 pluck samples		27.22	37.18	32.20	—	—	—	—

3. RESULTS

(a) *Environmental measurements*

During the behavioural observation period, tussocks were consistently taller than inter-tussock swards (variance ratio 1889, d.f. = 2,114, $p < 0.001$), and both sward types increased in height during the same period (effect of day: variance ratio = 49.3, d.f. = 2,114, $p < 0.001$). A significant sward type × day interaction was due to greater increase in the height of the tussock swards relative to the inter-tussock swards (variance ratio = 17.2, d.f. = 2,114, $p < 0.001$; table 1). Similarly, tussocks had consistently higher sward nitrogen content than inter-tussock swards throughout the behavioural observation period (variance ratio = 84.2, d.f. = 1,12, $p < 0.001$); however, the nitrogen content of both sward types decreased during the behavioural observation period (variance ratio = 8.96, d.f. = 2,12, $p < 0.001$). There was no sward type × day interaction effect on sward nitrogen content (variance ratio = 3.77, d.f. = 2,12, $p > 0.5$). The OMD of the swards followed a pattern similar to the nitrogen content with tussock swards being more digestible than inter-tussock swards and the digestibility of both sward types declined during the behavioural observation period (effect of sward type: variance ratio = 19.69, d.f. = 1,12, $p < 0.001$; effect of day: variance ratio = 20.32, d.f. = 2,12, $p < 0.001$; interaction: variance ratio = 0.91, d.f. = 2,12, $p > 0.1$). Mean overall proportional tussock availability from the beginning, middle and end of the behavioural observation period was 0.352 ± 0.0046, ranging from 0.361 at the start of the behavioural observation period to 0.347 at the end. Five hundred pluck samples from tussocks created 1.36 times more DM than the inter-tussock pluck samples (table 1).

(b) *Parasitology*

Once the animals started excreting parasite eggs in their faeces (three weeks after the sheep were moved on to the experimental pasture), the R-sheep had consistently lower FECs than the S-sheep throughout the experiment (figure 1). Three weeks after the behavioural observation period (i.e. time for parasite larvae ingested during the experiment to mature and excrete eggs), FECs of the resistant sheep were lower than the susceptible sheep (resistant $= 30 \pm 9.6$ epg and susceptible $= 245 \pm 77.6$ epg; deviance ratio = 18.67, d.f. = 1, $p < 0.001$). Tussock swards contained 17 times more infective parasite larvae compared with inter-tussock swards (deviance ratio = 25.73, d.f. = 1, $p < 0.001$; table 1).

(c) *Sheep sward selection and grazing behaviour*

Relative to tussock availability, both Perendale selection lines avoided grazing tussocks throughout the behavioural observations (figure 2). However, the two Perendale selection lines differed in their grazing decisions with the R-sheep grazing the parasite-contaminated tall tussock patches to a lesser degree than the S-sheep ($W = 86.1$, d.f. = 1, $p < 0.001$; figure 2). Tussock use did not vary between days ($W = 13.4$, d.f. = 8, $p > 0.05$) and there was no interaction ($W = 4.30$, d.f. = 8, $p > 0.5$). The increased use of tussocks by parasite-susceptible animals was associated with a reduction in bite rate (R-sheep = 0.793 ± 0.013 bites per second versus S-sheep = 0.709 ± 0.013 bites per second: $W = 21.8$, d.f. = 1, $p < 0.001$) and movement rate (R-sheep = 0.1000 ± 0.0028 steps per second versus S-sheep = 0.084 ± 0.0028 steps per second: $W = 16.4$, d.f. = 1, $p < 0.001$). Both bite rate and step rate varied between days, but no consistent trend throughout the behavioural observation period was present (bite rate: $W = 2.84$, d.f. = 8, $p < 0.01$; step rate: $W = 3.56$, d.f. = 8, $p < 0.001$).

4. DISCUSSION

Our aim was to test whether genetically resistant sheep avoid parasites to a greater extent than susceptible sheep when grazing. The first step was to ensure that the structure of the sward in the experimental plot replicated the typical natural tall (tussock) versus short (inter-tussock) sward patch mosaic present in extensive grazing and natural systems (Hutchings *et al.* 2002*b*). At the start of the period of behavioural observations, tussock swards were approximately three times taller than inter-tussock swards. Both the tussock and inter-tussock swards increased in height during the behavioural observation period, suggesting that forage resources were not limiting and enabling animals to freely discriminate when selecting a diet in relation to the heterogeneous sward structure without pressure from nutritional restriction. This mosaic contained a nutrition versus parasitism trade-off in that a grazing animal could increase its nutrient intake rate

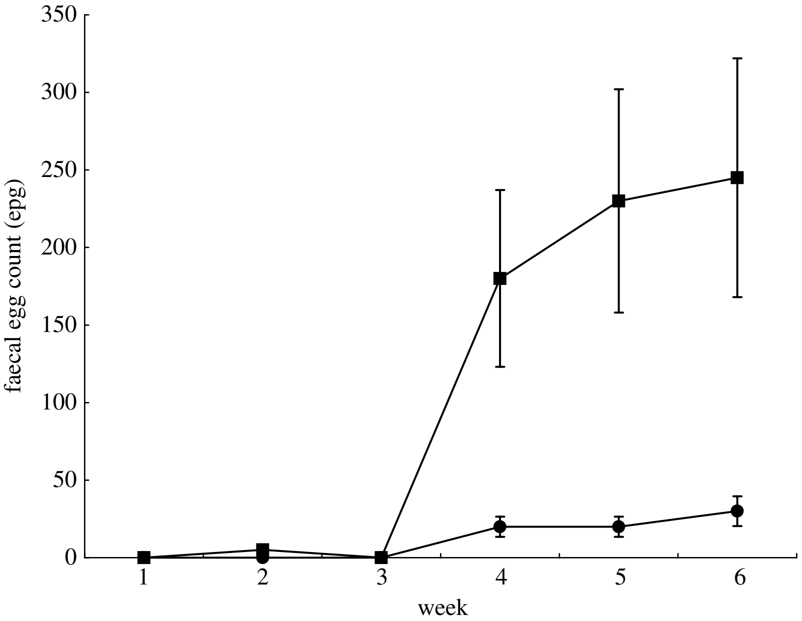

Figure 1. Faecal egg counts of the resistant (filled circles) and the susceptible (filled square) sheep. Values are predicted means and s.e. of means from the GLM analysis. Behavioural observations occurred in weeks 1 and 2.

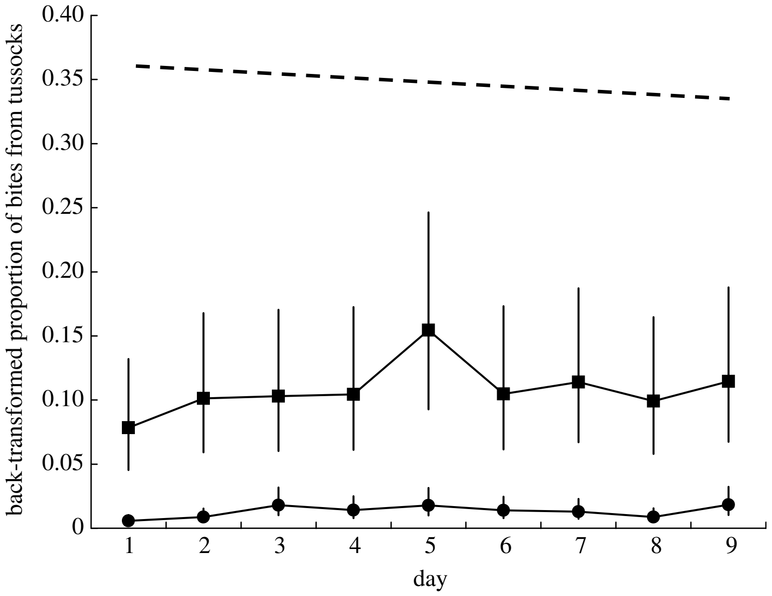

Figure 2. Tussock use by the resistant (filled circles) and susceptible (filled squares) sheep during the 9-day period of the behavioural observations. Back-transformed mean proportions of bites from tussocks are presented with 95% confidence limits. Proportional tussock availability by area is shown by the dashed line.

through a combination of increased forage intake rate (32%) and increased nitrogen content of forage (41%) by selecting tussocks for grazing; however, in the process, they would also contact some 17 times more parasites (table 1).

When faced with this trade-off, the two Perendale selection lines differed in their grazing decisions, with the R-sheep grazing the parasite-contaminated tall tussock patches to a lesser degree than the S-sheep. However, relative to tussock availability (35% by area), all animals consistently avoided the parasite-contaminated tall tussock patches throughout the experiment, which is consistent with previous studies (Hutchings *et al.* 2002*a*,*b*). Indeed the R-sheep made almost no attempt to graze the tussock sward patches, thereby avoiding the greatest concentrations of parasites in their environment. The increased grazing of the tall tussocks by parasite-susceptible animals was associated with a lower bite rate, which is consistent with the effects of sward height on bite rate (Black & Kenney 1984; Hutchings *et al.* 2002*a*). Once the shedding of parasite eggs in faeces had started, the R-sheep had consistently lower FECs than the S-sheep throughout the experiment. Three weeks after the behavioural observation period (i.e. time for parasite larvae ingested during the experiment to mature and excrete eggs), FECs of the resistant sheep were lower than the susceptible sheep. Avoiding grazing faecal-contaminated swards results in animals avoiding the greatest concentrations of parasites (Michel 1955); however, contacting parasites and parasitism in grazing systems are almost inevitable, and so improved immune function in the R-sheep would also be expected to place them at an advantage over the S-sheep (Hutchings *et al.* 2003). The reduced parasite challenge and increased ability of the R-sheep to fight parasitism through immune function would both act to reduce their FEC relative to the S-sheep. As such the R-sheep may be seen as having two ways to combat parasites: avoiding parasites where possible, but using improved immune function to fight when necessary; prevention; and cure.

Using FEC during natural parasite challenge may have selected for parasite avoidance behaviours that reduce their parasite exposure/challenge rather than simply immune function. The avoidance of faeces, and the parasites therein, has been shown to be a hard-wired response in sheep (Hutchings *et al.* 2003). R-sheep may thus avoid faeces to a greater extent than S-sheep. However, we cannot rule out the possibility that the selected-for behavioural trait was sward height or nitrogen content (affecting sward darkness), both representing visual cues used by herbivores to maximize nutrient intake and being confounded with the distribution of parasites. Irrespective of the cue used, the increased avoidance of the parasite-rich tussock swards prevented the R-sheep from taking advantage of the tussock's nutritional benefits (increased nitrogen and forage intake rates) that could have been used to enhance immune responses and/or for growth (production; Houdijk *et al.* 2005). These nutritional penalties associated with the grazing strategy of the R-sheep may help explain the negative genetic correlations between production traits (e.g. growth and fleece weight) and parasite resistance in the Perendale lines (Morris *et al.* 2005).

The reduced productive performance of R-Perendale sheep has been seen as counterintuitive, as sheep with an increased ability to combat parasites through improved immunity are expected to be freed from the nutrient losses associated with parasitism and, therefore, perform better than the S-sheep. R-sheep strongly and consistently avoiding grazing the swards that offer the best nutrition may explain their relatively poor performance and suggests that they adopt an overly cautious strategy for combating parasites. While there are examples of parasite-resistant sheep being associated with improved performance in agricultural and natural systems (Bishop *et al.* 1996; Coltman *et al.* 2001), differences in the selection histories and pressures between these studies complicate any comparison with the current study. However, in using FEC from natural challenge, livestock breeding programmes around the world may include traits that are potentially antagonistic to their production goals.

Previously, the results of livestock parasite-resistance selection experiments have been interpreted in relation to a host's ability to combat disease through its immune function. Our findings suggest that parasite avoidance behaviours may account for some of the variation in FECs between resistant and susceptible sheep lines, especially where animals were selected under natural grazing conditions. The relative contribution of improved immunity versus reduced parasite exposure in R-sheep having lower FEC than S-sheep at the end of the experiment is unknown. The early experimental selection procedures could only select for improved immunity in the R-sheep, which have only recently been shown to differentially express genes involved in immune function, and suggests that this difference in immune function between the selection lines remained, during the period of selection, under natural challenge. The selection criteria (i.e. using FEC scores) remained constant throughout both selection periods (experimental and natural challenge); however, selection for parasite avoidance can only have occurred during the natural challenge selection period. It is at least theoretically possible that the genes associated with the grazing behaviour were close to those for immune function and were co-selected. It would seem more likely that behaviours enhancing the difference in FEC scores between the selection lines were included during the natural parasite challenge selection period (i.e. the selection acted on between individual variations in parasite avoidance, e.g. level of faecal avoidance). While we recommend caution when extrapolating from small-scale experiments, this experiment suggests that using an indirect measure of a target trait is not necessarily straightforward, especially when used in a complex natural system. However, this experiment also highlights the potential for livestock host behaviour to contribute to parasite control and is the first evidence suggesting that animals can be bred to avoid disease. The combination of increased parasite avoidance and improved immunity in genetically resistant animals, selected in this long-term study using natural parasite challenge, is highly effective at reducing parasite burdens, but it may have knock-on effects that are antagonistic to overall production goals.

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