Host immune responses are necessary for density dependence in nematode infections

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SUMMARY

Nematode infections are subject to density-dependent effects on their establishment, survivorship and fecundity within a host. These effects act to regulate and stabilize the size of nematode populations. Understanding how these density-dependent effects occur is important to guide the development of control strategies against parasitic nematodes and the diseases that they cause. These density-dependent effects have been hypothesized to result from intraspecific competition between parasites for limited resources or from the action of host immune responses. However, no specific evidence exists to distinguish between these two hypotheses. We find that in nematode (*Strongyloides ratti*) infections, density-dependent effects on parasite establishment, survivorship and fecundity are mediated by the host immune response. These density-dependent effects are only observed late in primary infections and no density-dependent effects are observed in infections in immuno-compromised animals. We find no evidence for intraspecific competition between parasites in experimental infections over a range of doses that encompasses all that is observed in natural infections. We conclude that density-dependent effects due to the immune response will act to regulate *S. ratti* infections before competition for space or nutrients within the host gut ever occurs.

Key words: regulation, population dynamics, epidemiology, nematodes, parasites, ecology.

INTRODUCTION

Nematode infection is a ubiquitous feature of wild animal, domestic livestock and human populations (Anderson & May, 1992; Sousa, 1994). These infections are usually endemic, with all individuals in a host population exposed to, and infected by, these parasites (Anderson & May, 1992). Nematode infections generally cause morbidity which, in humans, can impair childhood growth and development (Stephensen, 1999). Similarly, nematodes of domestic livestock cause major productivity and economic losses to the agricultural industry (Barnes, Dobson & Barger, 1995) and morbidity effects of parasitic nematodes in natural host populations can exacerbate mortality due to starvation (Gulland, 1992).

Parasitic nematode populations are subject to density-dependent effects on major life-history traits, namely establishment, survivorship and fecundity (Anderson & Michel, 1977; Keymer, 1982; Scott & Lewis, 1987; Quinnell, Medley & Keymer, 1990; Anderson & May, 1992). These density-dependent effects regulate and stabilize nematode populations but the basis of these density-dependent effects remains controversial. Host immune responses are known to reduce the establishment, survivorship and fecundity of nematode infections *per se* (Michel & Sinclair, 1969). Density-dependent effects on these traits have also been hypothesized to be due to (innate or adaptive) immune responses whose severity on individual parasites increases with parasite density (Anderson & Michel, 1977; Keymer, 1982; Scott & Lewis, 1987; Quinnell *et al*. 1990; Anderson & May, 1992). However, intraspecific competition between parasites for resources within the host gut has also been hypothesized to cause these density-dependent effects. Previous work has not separated the effect of intraspecific competition from that of the host immune response. For example, restricting nutrients to nematodes in the gut in an attempt to increase intraspecific competition also restricts nutrients to the host (Quinnell *et al*. 1990), thereby stressing the host and inducing a steroid response that compromises the host immune system (Facuci, 1979). Similarly, treatment of hosts with corticosteroids to reduce the effect of the immune response on parasites (Michel & Sinclair, 1969) also has systemic effects on host metabolism and leads to increased appetite and food intake (Baxter & Rousseau, 1979), thereby reducing any effects of intraspecific competition.

Separating the effects of intraspecific competition for resources from those of the host immune response is essential to understand nematode epidemiology. This is because density-dependent effects due to intraspecific competition for resources will depend

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only on current infection levels, whereas immune-mediated, density-dependent effects will depend on infection history (Quinnell *et al*. 1990; Anderson & May, 1992). Epidemiological models now exist that incorporate the effect of acquired immunity and infection history (Anderson & May, 1992; Woolhouse, 1992, 1998; Grenfell *et al*. 1995). However, the use of these models as accurate, predictive tools to develop control strategies against parasitic nematodes is hampered by a lack of empirical data concerning the relative effects of intraspecific competition and host immune responses on the regulation of nematode populations. Here we use an experimental rat–nematode system to investigate the roles of the host immune response and intraspecific competition on the generation of density-dependent effects on parasite fitness.

MATERIALS AND METHODS

Study system

The nematode *Strongyloides ratti* is a natural parasite of rats. Infective 3rd-stage larvae (iL3s) infect percutaneously and developing larvae migrate to the host gut, where they develop into adult, parasitic females only. Reproduction commences 4–5 days post-infection (Dawkins, 1989). Eggs are produced by mitotic parthenogenesis and are passed with the faeces into the external environment (Viney, 1994). Experimental infections of *S. ratti* in rats are naturally cleared from rats after 1–2 months, coincident with the development of an adaptive, anti-*S. ratti* immune response (Bell, Adams & Gerb, 1981; Kimura *et al*. 1999).

Measuring parasite fitness

We analysed 3 parasite fitness traits; establishment, survivorship and fecundity. We define establishment as the probability that an iL3 reaches the host gut and develops into a parasitic female at 5 days post-infection (p.i.); survivorship as the probability that a parasitic female survives to time t p.i. and fecundity as the average number of viable eggs produced by a parasitic female at time t p.i. We also determined the reproductive output of infections. Reproductive output is the total number of viable larvae produced by an infection at time t, and is the product of the number of adult female worms in the gut and their average fecundity at time t.

Rats were infected by inoculation with a known dose of *S. ratti* iL3s from line ED321 Heterogonic throughout (Harvey *et al*. 2000). The reproductive output of an infection was determined by counting the number of worms present in cultures of faeces collected from rats overnight (Gemmill, Viney & Read, 1997). The number of adult worms in the gut of a host was determined directly by counting worms in the gut of sacrificed animals from which food had been previously withdrawn for 16 h (Gemmill *et al*. 1997). The fecundity of a rat's infection is the reproductive output divided by the number of parasitic females in the rat.

Establishment, survivorship and fecundity were analysed using generalized linear modeling (GLM) assuming a negative binomial error distribution with dispersion parameter k (Wilson & Grenfell, 1997). Establishment and survivorship were analysed in one GLM with the number of parasitic females as the response variable with the natural log of inoculating dose as an offset variable to generate the establishment and survivorship probabilities. GLMs make maximum use of our factorial designs since, for example, estimates of establishment are based not only on worm counts from animals sacrificed on day 5 but also on animals at all subsequent time-points. This form of GLM analysis is analogous to standard k-factor analysis (Begon, Harper & Townsend, 1996), since the GLMs will analyse changes in log survivorship and fecundity, but it has the advantage of accommodating the overdispersed nature of parasite distributions (Wilson & Grenfell, 1997). We note, however, that this approach does not *a priori* set establishment and survivorship at less than 1 as a binomial error distribution would. However, a binomial distribution, even with a scale parameter, is prone to Type II errors (Crawley, 1993; McCullagh & Nelder, 1989) and would hence reduce the power to detect biologically significant effects. Significance of explanatory variables and their interactions were determined using deletion testing, with significance of a term determined by the log-likelihood ratio test, i.e. referring twice the log-likelihood difference for nested models to a χ^2 distribution (McCullagh & Nelder, 1989). This process of deletion testing was used to construct minimal models from significant explanatory variables and their interactions. Where an interaction term was found to be significant, the lower order terms involved in that interaction were also retained (Crawley, 1993). Fecundity was similarly analysed but used the reproductive output of an infection as the response variable and the number of parasitic females as the offset variable. Density is the inoculating dose given to a host.

Experiment 1. Density-dependent effects on S. ratti *infections*

Six groups of 6, approximately 100 g, female Wistar rats (Bantim and Kingman, UK) were infected with 10, 30, 100, 300, 1000 or 2000 iL3s. This range of doses encompasses all that are observed in natural infections (Fisher & Viney, 1998). Rats were faecally sampled (above) and sacrificed during the experiment to determine establishment and fecundity at

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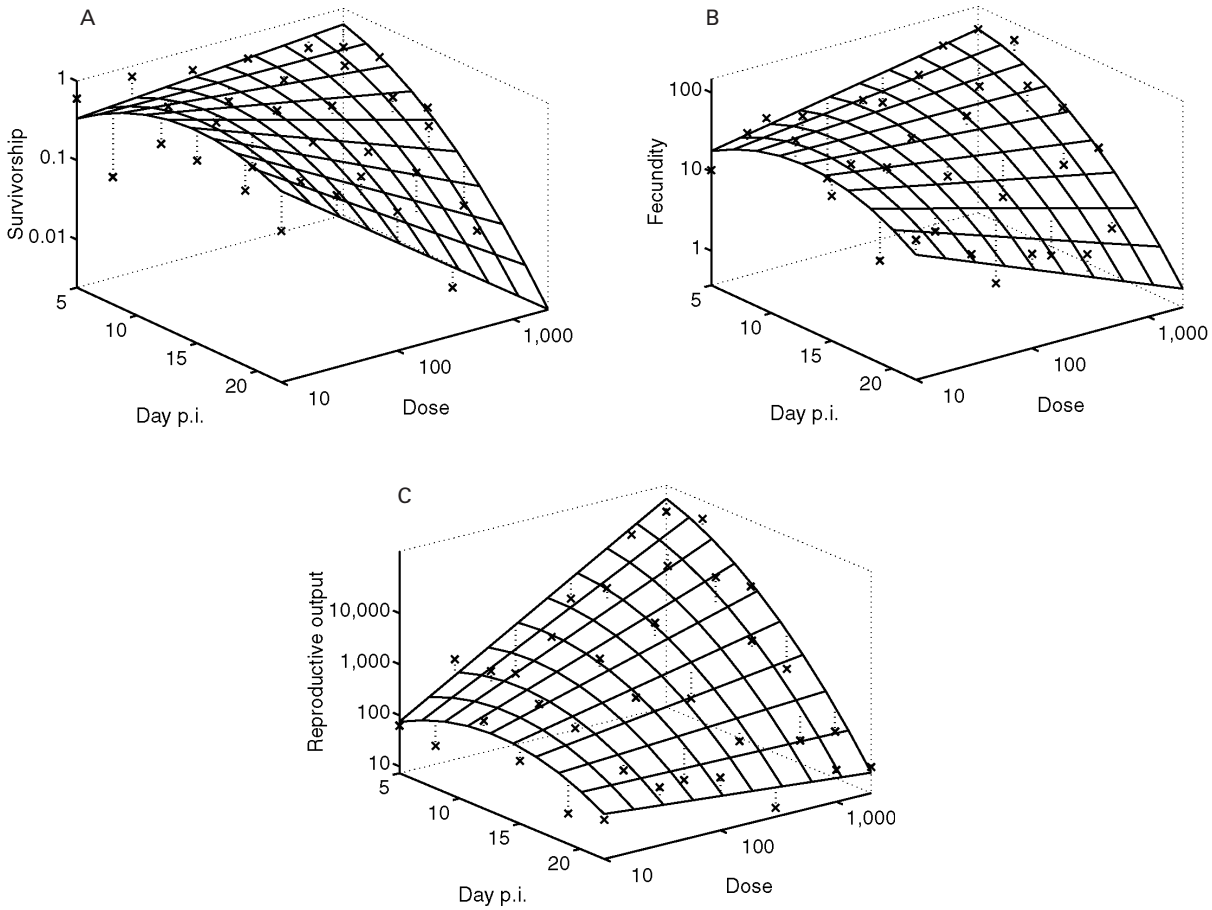


Fig. 1. Density-dependent effects on *Strongyloides ratti* infections. Data (x) from 6 groups of 6 rats were fitted to a GLM (mesh) to show (A) density-dependent effect of inoculating dose on parasite establishment and survivorship through time. Establishment is the survivorship of infective L3s to the onset of patency, i.e. 5 days p.i. (B) Density-dependent effect of inoculating dose on fecundity through time. (C) Density-dependent effect of inoculating dose on the reproductive output of the infection through time, where reproductive output is the number of viable eggs produced by each rat at each time-point.

day 5 p.i. and survivorship and fecundity at days 8, 12, 15, 19 and 22 p.i.

Experiment 2. Effect of host immune responses on density-dependent effects on S. ratti *infections*

Twelve, approximately 100 g, female Wistar rats and 12, 100–200 g, female immuno-compromised *rnu/rnu* (nude) rats (Charles Rivers) were infected with 10, 30, 80, 240, 700 or 2000 infective L3s. Nude rats are homozygous for a mutation and as such lack a functional thymus and so are unable to mount a T-cell dependent, adaptive immune response (Gemmill *et al*. 1997). The dynamics of *S. ratti* infection in Wistar rats and *rnu/+* heterozygotes are indistinguishable, indicating that the difference between nude (*rnu/rnu*) and Wistar rats with respect to *S. ratti* infection is due solely to the effects of the *rnu* mutation on the immune response of nude rats (Gemmill *et al*. 1997). The reproductive output of the infections was determined on days 5 and 20 p.i. and the number of parasitic females determined on day 22 p.i. Day 22 p.i. was chosen since it was the latest time-point analysed in Exp. 1. Separate GLMs for Wistar and nude rats were used to determine the density-dependent effect of inoculating dose on survivorship, fecundity and reproductive output in these animals. The slope of a regression of reproductive output against inoculating dose will be one in the absence of any density-dependent effects on reproductive output. The slope of a regression of survivorship or fecundity against inoculating dose will be zero in the absence of density-dependent effects on these measures.

Experiment 3. Effect of prior exposure on density-dependent effects on S. ratti *infections*

On day 0, 4 groups of 16, approximately 100 g, female Wistar rats were given a primary dose of 0, 1, 10 or 100 iL3s. On days 11 and 12 p.i. all animals were dosed orally with 70 μl of 17·6% (w/w) thiabendazole (Sigma, UK) suspended in sunflower oil (Gemmill *et al*. 1997). On day 14 p.i., 4 animals

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from each group were given a secondary dose of 10, 60, 360 or 2000 iL3s. Thus, the secondary infection was initiated 48 h after thiabendazole treatment. Previous toxicity studies on rats show that virtually all thiabendazole is expelled from rats within 48 h (Edwards, Ferry & Temple, 1991). The establishment and fecundity of the secondary infection was measured on day 21 p.i. and its survivorship and fecundity was measured on days 24, 28 and 31 p.i., as described above.

RESULTS

Experiment 1. Density-dependent effects on S. ratti *infections*

The establishment, survivorship and fecundity of the infections established with a range (10–2000 iL3s) of infective doses are shown in Fig. 1, with the corresponding minimal models shown in Table 1.

There was no effect of the size of the inoculating dose on establishment (LN DOSE = 0·119 ± 0·140, $\chi^2_{v=1} = 0{\cdot}92$, N.S., Table 1). As infections progressed in time there was a negative, density-dependent effect of inoculating dose on parasite survivorship (TIME · LN DOSE = −0·068 ± 0·014, $\chi^2_{v=1} = 29{\cdot}83$, $P < 0{\cdot}001$, Table 1). Thus, the probability of a parasitic female establishing in the gut is independent of size of inoculating dose. However, for a parasitic female that does establish, its survivorship decreases as the size of inoculating dose increases.

At the start of the infection there was a weak, positive density-dependent effect of inoculating dose on fecundity (LN DOSE = 0·271 ± 0·109, $\chi^2_{v=1} = 7{\cdot}14$, $P < 0{\cdot}01$, Table 1). As the infection progressed a strong, negative density-dependent effect on fecundity developed (TIME · LN DOSE = −0·050 ± 0·013, $\chi^2_{v=1} = 14{\cdot}83$, $P < 0{\cdot}001$, Table 1). These density-dependent effects on fecundity can be explained by either the size of the inoculating dose or the number of parasitic females in the gut. Thus, inoculating dose and number of parasitic females are correlated measures and explain a similar proportion of the deviance in models of parasite fecundity. It is therefore difficult to determine whether parasite fecundity depends more on the current parasite burden or on the number of parasites that a host has experienced in an infection. However, since slightly more deviance is explained by inoculating dose than by the number of parasitic females we provide this in the preferred minimal model. We note that there is no significant increase in deviance explained following either addition of number of parasitic females to a model containing inoculating dose ($\chi^2_{v=2} = 1{\cdot}80$, N.S.) or addition of inoculating dose to a model containing number of parasitic females ($\chi^2_{v=2} = 5{\cdot}83$, N.S.). In summary, we only observed negative density-dependent effects that reduced the survivorship and fecundity of *S. ratti* infections late in an infection.

Table 1. Density-dependent effects on *Strongyloides ratti* infections

Term	Coefficient ± S.E.	χ^2	*P* value
Establishment and survivorship			
CONSTANT	−1·364 ± 0·808		
TIME*	0·411 ± 0·117	11·76	< 0·001
TIME²†	−0·013 ± 0·005	7·03	< 0·01
LN DOSE	0·119 ± 0·140	0·92	N.S.
TIME · LN DOSE	−0·068 ± 0·014	29·83	< 0·001
($2 \times$ log likelihood = $3{\cdot}79 \times 10^4$, $k = 2{\cdot}16$, response variable = PARASITIC FEMALES, offset variable = LN DOSE)			
Fecundity			
CONSTANT	2·280 ± 0·614		
TIME*	0·329 ± 0·104	9·02	< 0·01
TIME²†	−0·014 ± 0·004	9·98	< 0·01
LN DOSE	0·271 ± 0·109	7·14	< 0·01
TIME · LN DOSE	−0·050 ± 0·013	14·83	< 0·001
($2 \times$ log likelihood = $3{\cdot}99 \times 10^6$, $k = 2{\cdot}84$, response variable = REPRODUCTIVE OUTPUT, offset variable = LN PARASITIC FEMALES)			
Reproductive output			
CONSTANT	1·235 ± 0·695		
TIME*	0·509 ± 0·102	19·58	< 0·001
TIME²†	−0·019 ± 0·005	15·95	< 0·001
LN DOSE‡	1·338 ± 0·124	8·08	< 0·01
TIME · LN DOSE	−0·091 ± 0·012	36·43	< 0·001
($2 \times$ log likelihood = $3{\cdot}99 \times 10^6$, $k = 2{\cdot}01$, response variable = REPRODUCTIVE OUTPUT, no offset variable)			

* TIME is fitted to the model from the onset being patency, i.e. TIME = 0 is equivalent to 5 days p.i.

† TIME² was fitted to increase the deviance explained by the model and thereby to increase the accuracy of estimates of other terms.

‡ The significance of the DOSE term for reproductive output is given in comparison to a model with the coefficient of DOSE equal to 1, i.e. no density-dependent effect on reproductive output at the start of the infection. All terms are continuous variables, hence the significance of all lower order terms can be determined by deletion testing since none are marginal to higher order terms (i.e. no lower order term is necessary to define a higher order term). LN, natural log.

Experiment 2. Effect of immune responses on density-dependent effects on S. ratti *infections*

We hypothesized that the host immune response against an *S. ratti* infection generated the density-dependent effects observed in Exp. 1. We tested this by comparing *S. ratti* infections, over a range of inoculating doses (10–2000 iL3s per rat), in immunocompetent rats and in immuno-compromised, nude rats. At the start of the infections in both immunocompetent and immuno-compromised rats there was a significant difference in the intercept of the two groups with the *y*-axis, possibly reflecting an inherent physiological difference between Wistars and nude rats. However, at the start of the infections, the

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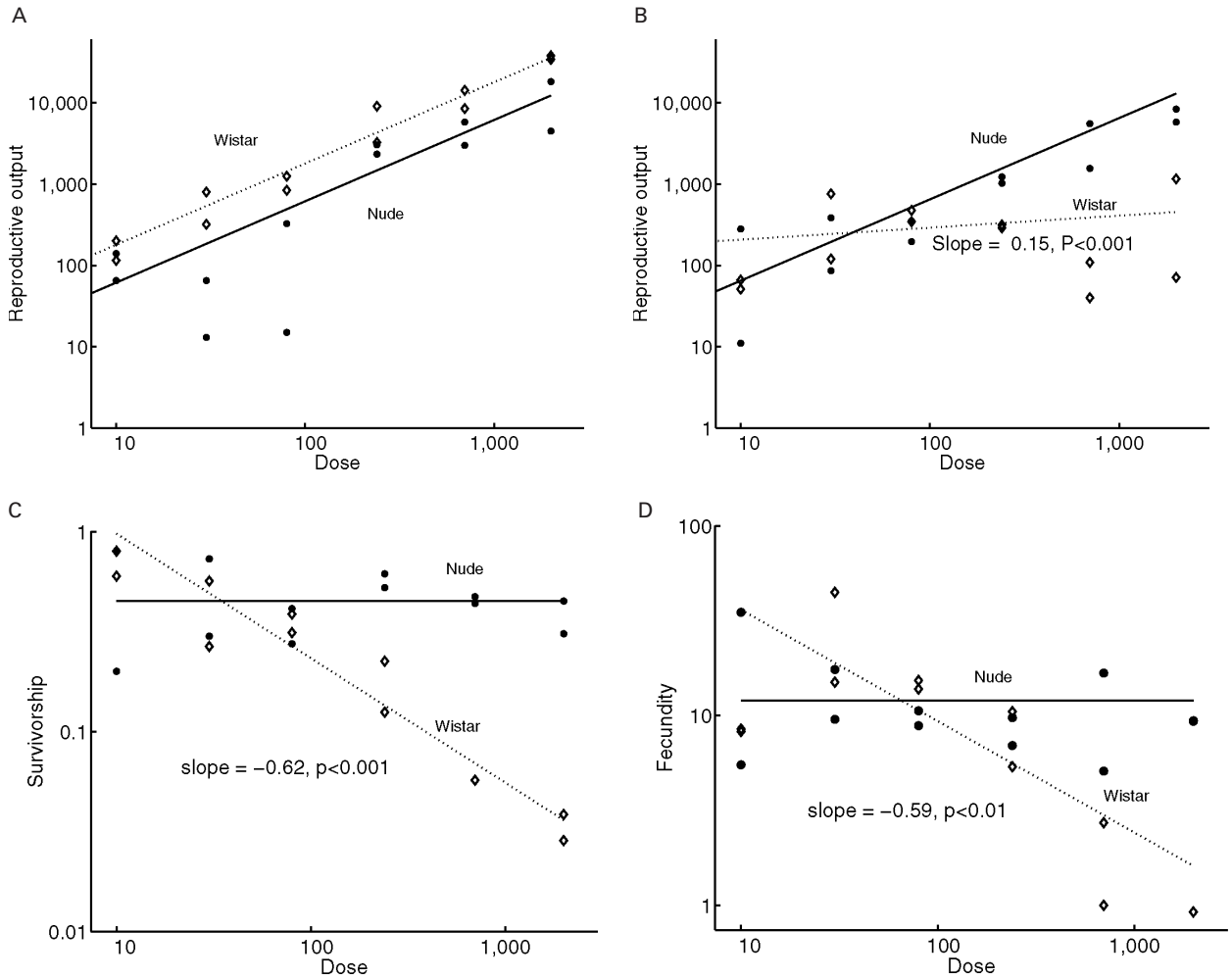


Fig. 2. Effect of host immune responses on density-dependent effects on *Strongyloides ratti* infections. (A) Reproductive output on day 5 p.i. (B) reproductive output on day 20 p.i., (C) survivorship on day 22 p.i. and (D) fecundity on day 22 p.i. for nude, immuno-compromised rats (●) and Wistar, immuno-competent rats (◇) infected with 10–2000 *S. ratti* iL3s. Regression lines are given by a GLM with a negative binomial for nude rats (———) and Wistar rats (·····). Values of the slope are shown where they are found to be significantly different from 1, in (A) and (B), or from 0, in (C) and (D).

reproductive output of infections in both immuno-competent and immuno-compromised animals increased linearly with inoculating dose (Fig. 2A), indicating a lack of density-dependent effects on reproductive output at the start of the infection in either group. As seen previously, later in the infection in the immuno-competent, control animals there was a strong, negative density-dependent effect of dose on the reproductive output of the infection (LN DOSE $= 0{\cdot}148 \pm 0{\cdot}175$, $\chi^2_{v=1} = 13{\cdot}02$, $P < 0.001$, Fig. 2B), which was due to the combination of density-dependent effects of dose on parasite survivorship (LN DOSE $= -0{\cdot}623 \pm 0{\cdot}054$, $\chi^2_{v=1} = 29{\cdot}46$, $P < 0{\cdot}001$, Fig. 2C) and on fecundity (LN DOSE $= -0{\cdot}587 \pm 0{\cdot}141$, $\chi^2_{v=1} = 8{\cdot}66$, $P < 0{\cdot}01$, Fig. 2D). In contrast, later in infections in immuno-compromised, nude rats the reproductive output of the infection remained linear with dose (Fig. 2B). This was due to the absence of any density-dependent effects on parasite survivorship or on fecundity (Fig. 2C and D).

In summary, we observed density-dependent effects late in infections in immuno-competent animals, as in Exp. 1, but did not observe any density-dependent effects at any time in immuno-compromised animals.

Experiment 3. Effect of prior exposure on density-dependent effects on S. ratti *infections*

We investigated the effect of immunological memory on these observed density-dependent effects by determining the effect of the magnitude of prior exposure to *S. ratti* (0–100 iL3s per rat) on the generation of density-dependent effects in a subsequent, secondary infection (10–2000 iL3s per rat) 14 days after the primary dose was given. Minimal models for establishment and survivorship and for fecundity are presented in Table 2 and the fit of these models to the observed data are presented in Fig. 3. We find that there were density-dependent effects on the secondary infection due to the dose of the prior

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Table 2. Effect of prior exposure on density-dependent effects on *Strongyloides ratti* infections

Term	Coefficient ± S.E.	χ^2	P value
Establishment and survivorship			
CONSTANT	−0·511 ± 0·404		
TIME*	0·227 ± 0·088	7·23	< 0·01
TIME²†	−0·014 ± 0·007	4·62	< 0·05
LN SECONDARY DOSE	−0·040 ± 0·069	0·37	N.S.
SQRT PRIMARY DOSE	0·177 ± 0·058	9·43	< 0·01
SQRT PRIMARY DOSE · LN SECONDARY DOSE	−0·044 ± 0·010	18·95	< 0·001
TIME · LN SECONDARY DOSE	−0·041 ± 0·010	16·15	< 0·001
TIME · SQRT PRIMARY DOSE	−0·015 ± 0·005	10·37	< 0·01
($2 \times$ log likelihood $= 5{\cdot}11 \times 10^4$, $k = 4{\cdot}96$, response variable = PARASITIC FEMALES, offset variable = LN SECONDARY DOSE)			
Fecundity			
CONSTANT	1·250 ± 0·386		
TIME*	0·222 ± 0·061	14·21	< 0·001
LN SECONDARY DOSE	0·160 ± 0·072	5·62	< 0·05
TIME · LN SECONDARY DOSE	−0·026 ± 0·011	6·12	< 0·05
($2 \times$ log likelihood $= 9{\cdot}09 \times 10^5$, $k = 2{\cdot}52$, response variable = REPRODUCTIVE OUTPUT, offset variable = LN PARASITIC FEMALES)			

* TIME is fitted to the model from the onset being patency, i.e. TIME = 0 is equivalent to 5 days p.i.

† TIME² was fitted to increase the deviance explained by the model and thereby to increase the accuracy of estimates of other terms. All terms are continuous variables, hence the significance of all lower order terms can be determined by deletion testing since none are marginal to higher order terms (i.e. no lower order term is necessary to define a higher order term). LN, natural log; SQRT, square root.

exposure and due to the dose of the secondary infection itself. Thus, as with the primary infections (and Exp. 1) there are density-dependent effects of secondary dose on the survivorship and fecundity of the secondary infection (TIME · LN SECONDARY DOSE $= -0{\cdot}041 \pm 0{\cdot}010$, $\chi^2_{v=1} = 16{\cdot}15$, $P < 0{\cdot}001$ and TIME · LN SECONDARY DOSE $= -0{\cdot}026 \pm 0{\cdot}011$, $\chi^2_{v=1} = 6{\cdot}12$, $P < 0{\cdot}05$, Table 2 for survivorship and fecundity, respectively).

Prior exposure decreased the establishment of a secondary infection. This effect is seen both as a single effect and as an interaction with the secondary dose (SQRT PRIMARY DOSE $= 0{\cdot}117 \pm 0{\cdot}058$, $\chi^2_{v=1} = 9{\cdot}43$, $P < 0{\cdot}01$; SQRT PRIMARY DOSE · LN SECONDARY DOSE $= -0{\cdot}044 \pm 0{\cdot}010$, $\chi^2_{v=1} = 18{\cdot}95$, $P < 0{\cdot}001$, Table 2). These effects of the prior exposure are most parsimoniously explained quantitatively (size of primary dose) rather than qualitatively (previous infection or no previous infection). A square root transformation (SQRT) of PRIMARY DOSE was chosen for its simplicity and its goodness of fit (a log + 1 transformation gave similar results but explained slightly less deviance). Prior exposure also quantitatively decreased the survivorship of a secondary infection, observed as an interaction of prior exposure and time (TIME · SQRT PRIMARY DOSE $= -0{\cdot}015 \pm 0{\cdot}005$, $\chi^2_{v=1} = 10{\cdot}37$, $P < 0{\cdot}01$, Table 2). There was no effect (quantitative or qualitative) of prior exposure on the fecundity of secondary infections.

In summary, we observed that previous exposure quantitatively decreased both the establishment and subsequent survivorship of parasitic females of a secondary infection.

DISCUSSION

In common with other host–nematode systems, *S. ratti* infections are regulated by density-dependent effects. We have dissected the fitness of *S. ratti*

Fig. 3. Effect of prior exposure on density-dependent effects on *Strongyloides ratti* infections. (A) Each subplot shows the observed survivorship (y axis) as a circle plotted against day p.i. from the beginning of the secondary infection (x axis) for all combinations of primary dose (vertical alignment) and secondary dose (horizontal alignment). Confidence limits (lines) are based on the negative binomial distribution with $k = 4{\cdot}96$. Some confidence limits include 0 and/or 1. Expected survivorship from the model shown in Table 2 is shown as a cross. (B) Each subplot shows the observed fecundity (y axis) as a circle plotted against day p.i. (x axis) for all combinations of primary dose (vertical alignment) and secondary dose (horizontal alignment). Confidence limits (lines) are based on the negative binomial distribution with $k = 2{\cdot}52$. Some confidence limits include 0. Expected fecundity from the model shown in Table 2 is shown as a cross.

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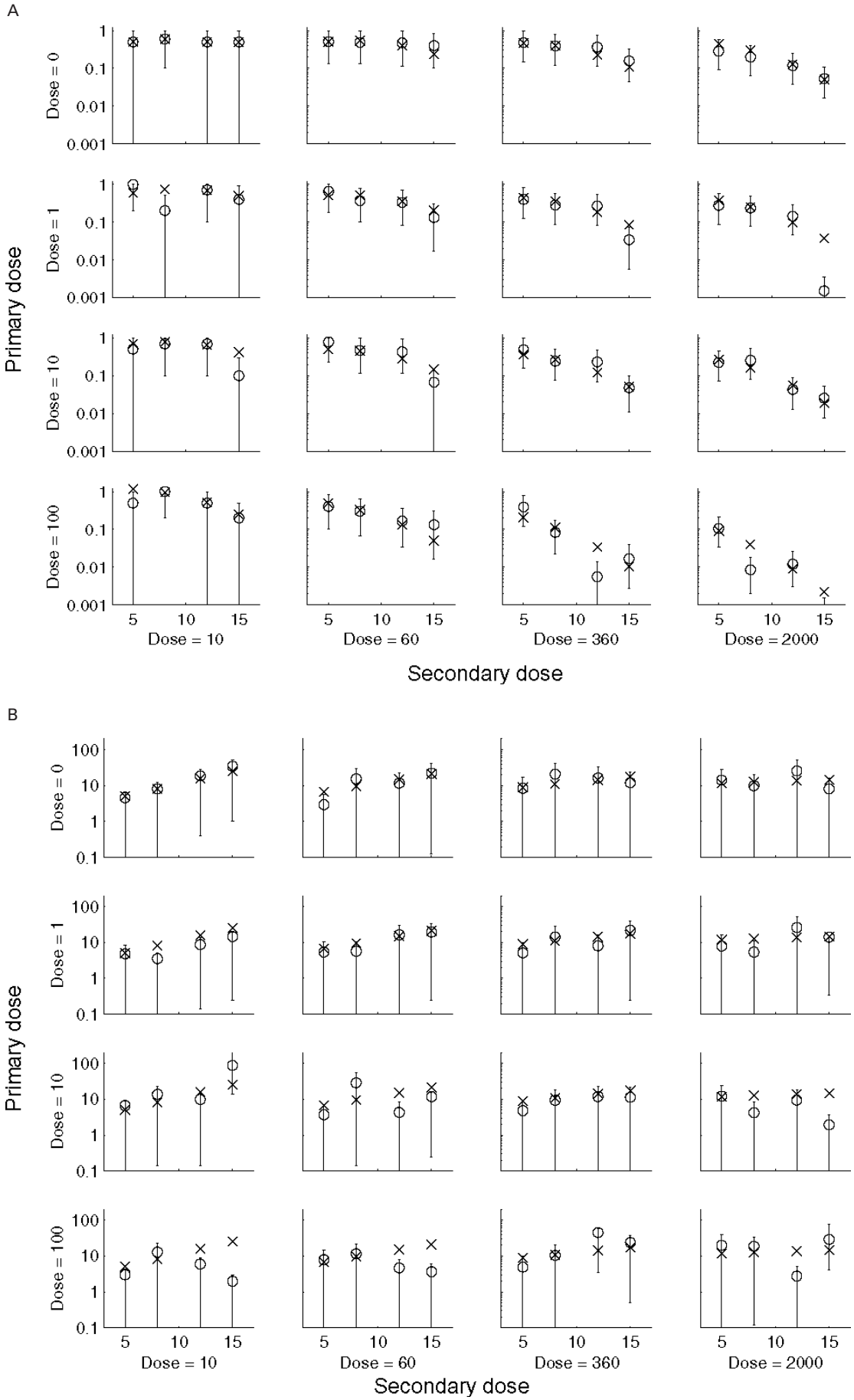


Fig. 3. For legend see opposite.

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infections into component traits; establishment, survivorship and fecundity. We have found that the survivorship, but not establishment, of *S. ratti* parasitic females is affected by the density of the inoculating dose an animal receives. Similarly, parasite fecundity is affected by the density of the inoculating dose, but only as an infection proceeds. The combination of these effects results in density-dependent effects on the reproductive output of *S. ratti* infections, which increase as infections progress in time. Our findings differ from observations of *Teladorsagia circumcincta* in lambs, where strong effects of numbers of worms in the gut are observed on fecundity but not on establishment or survivorship (Stear *et al*. 1995; Stear, Strain & Bishop, 1998). This may suggest that although density-dependent effects on nematode infections are widespread, the component fitness traits which are affected may vary between parasite and/or host species.

Our observations lead us to conclude that the density-dependent effects on *S. ratti* infections are mediated entirely by the host immune response. This is shown by the occurrence of density-dependent effects late in a primary infection, the enhancement of these effects by host prior exposure and the absence of density-dependent effects in immuno-compromised animals. We note that density-dependent effects mediated by the immune response may act either directly, through the action of immune effector cells and molecules on parasites, or indirectly, by immune-mediated gut pathology which is detrimental to parasites. The functional consequence of these two processes on parasite fitness are equivalent and we have not attempted to distinguish between them here.

The absence of density-dependent effects in immuno-compromised, nude rats demonstrates the absence of intraspecific competition over the range of infection doses used, which encompasses all that is observed in natural infections (Fisher & Viney, 1998). This suggests that density-dependent effects due to the immune response will act to regulate *S. ratti* infections before competition for space or nutrients within the host gut ever occur. These data are the first to separate successfully the effects of intraspecific competition from those of the immune response in helminth infections.

Our observations of the density-dependent effects of host prior exposure suggest that the fitness of a parasite infection in a host depends not only on the size of the current infection but also on the number of parasites to which a host has previously been exposed. For *S. ratti* infections, the level of prior exposure quantitatively reduces the establishment and survivorship of a subsequent infection. This is consistent with the view of natural infection processes in which a host is continuously exposed to parasites, which results in an accumulated immune experience such that the probability of an infection establishing and persisting decreases with host age. This scenario has been used to explain the observed decrease in intensity of infection with host age in natural helminth infections (Anderson & May, 1992; Woolhouse, 1998). Significantly, we do not find any effect of immunological memory on the fecundity of *S. ratti* infections. This shows that the parameters used in epidemiological models must be considered carefully as different components of parasite fitness may be affected differently by prior exposure compared with current exposure.

Results from the model system presented here demonstrate the potential of acquired immune responses to regulate nematode infections. However, it is unclear whether the acquired immune response will display the same role in helminth infections of humans. *S. ratti* females have the potential to live for more than a year in nude rats but are generally killed by the immune response within 2 months in immunologically normal laboratory rats (Bell *et al*. 1981; Gemmill *et al*. 1997). By contrast, many species of intestinal nematodes, filarial nematodes and other helminths can live for several years in humans (Anderson & May, 1992). The longevity of helminths in humans is probably a function of their ability to evade or modulate the host immune response, which would argue against a role for acquired immune responses in regulating helminth infections of humans (Behnke, 1987; Maizels *et al*. 1993). However, acquired immune responses provide the most likely explanation for the observed decrease in intensity of infection with host age in helminth infections of humans (Anderson & May, 1992). The peak intensity of these infections also occurs earliest in areas of high transmission, suggesting that the effects of these acquired immune responses are density dependent (Woolhouse, 1998).

Our results highlight the need for further work at the interface of immunology and ecology. A mechanistic understanding of the immunological pathways and processes by which hosts respond to and retain a memory of helminth infection is being realised (Maizels *et al*. 1993; Maizels & Holland, 1998; Ovington *et al*. 1999). However, the consequences of these immunological pathways and processes for the population dynamics of helminth infections are still unclear. In particular, we do not understand how helminth infection stimulates immunological processes in a density-dependent manner or how these immunological mechanisms achieve their density-dependent effects on different parasite fitness traits.

The aim of nematode control programmes is to reduce the disease caused by nematode infection. The severity of nematode-induced disease increases with the intensity of infection. For example, an infection of 50 hookworms causes only mild symptoms in humans but an infection of 1000 hookworms

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can cause severe disease (Behnke, 1987). Our findings suggest that immunological memory, elicited either by anti-nematode vaccines or by natural infection, will induce density-dependent effects on parasite establishment and survivorship. These density-dependent effects may, in turn, successfully control parasite-induced disease and hence improve human and animal health.

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