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Functional consequences of genetic diversity in *Strongyloides ratti* infections

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Parasitic nematodes show levels of genetic diversity comparable to other taxa, but the functional consequences of this are not understood. Thus, a large body of theoretical work highlights the potential consequences of parasite genetic diversity for the epidemiology of parasite infections and its possible implications for the evolution of host and parasite populations. However, few relevant empirical data are available from parasites in general and none from parasitic nematodes in particular. Here, we test two hypotheses. First, that different parasitic nematode genotypes vary in life-history traits, such as survivorship and fecundity, which may cause variation in infection dynamics. Second, that different parasitic nematode genotypes interact within the host (either directly or via the host immune system) to increase the mean reproductive output of mixed-genotype infections compared with single-genotype infections. We test these hypotheses in laboratory infections using genetically homogeneous lines of *Strongyloides ratti*. We find that nematode genotypes do vary in their survivorship and fecundity and, consequently, in their dynamics of infection. However, we find little evidence of interactions between genotypes within hosts under a variety of trickle- and single-infected infection regimes.

Keywords: immunity; ecology; genetics; nematode parasites; parasites

1. INTRODUCTION

Numerous genetic studies of parasitic nematodes have demonstrated the existence of substantial genetic diversity at polymorphic molecular markers (Anderson *et al.* 1998; Paterson & Viney 2000). These markers have been used to show that individual hosts harbour infrapopulations of nematodes (Anderson *et al.* 1998; Fisher & Viney 1998). However, the functional consequences of this genetic diversity within infrapopulations for the biology and epidemiology of parasitic nematode infections are poorly understood. *A priori*, genetic diversity will provide the basis for heritable, phenotypic variation between parasitic nematodes. Two types of phenotypic variation are likely to be of particular relevance. First, parasitic nematodes may vary in life-history traits, such as survivorship and fecundity (Pozio *et al.* 1992). Second, parasitic nematodes may vary in antigens available to their hosts and this, combined with antigen-specific immune effectors, may result in parasite genotype-specific immune effects (Read & Viney 1996). Two, not mutually exclusive, hypotheses about the functional consequences of genetic variation between individual parasitic nematodes can therefore be generated.

The first is that different parasitic nematode genotypes vary in life-history traits such as their within-host survivorship and *per capita* fecundity. These life-history traits are major determinants of the infection dynamics of parasitic nematodes, as parasite survivorship will strongly affect the persistence of an infection within a host and parasite fecundity will strongly affect transmission (Anderson & May 1992). Such variation in life-history traits is also likely to have important population-level consequences. Thus, variation between hosts in their level of exposure and/or susceptibility can act to stabilize the long-term dynamics of parasite populations (Anderson & May 1978). Variation in parasite life-history traits has received relatively little attention, but may have a similar role in stabilizing the dynamics of parasite populations. Some support for the hypothesis that genetic variation for parasite life-history traits exists is provided by the observations that different isolates of *Trichinella* exhibit different dynamics of infection (reviewed in Wakelin & Goyol 1996). For example, *Trichinella* isolates from Arctic wolverines and polar bears in the Arctic showed a lower reproductive output than isolates derived from rodents in temperate regions (Belosovic & Dick 1979; Chadee & Dick 1982). However, reproductive output was assayed in laboratory mice and so this difference may represent variation in host specificity rather than variation in life-history traits representative of a natural infection within a single-host species. Parasite isolates are also likely to adapt to laboratory conditions and to passage in laboratory animals (Read & Viney 1996) and may therefore not be representative of genotypes occurring naturally in the wild.

The second hypothesis is that parasitic nematodes of different genotypes interact within the host. A likely mechanism for any such interaction is via genotype-specific host immune responses (Read & Viney 1996), which are elicited by, or effective against, specific nematode genotypes. This has received little attention although there is limited evidence from *Trichinella*, where genotype-specificity of host immune responses has been observed. Thus, antigen preparations from homogenized muscle larvae derived from one isolate (London) protected laboratory mice against subsequent challenge from both itself and another isolate (Spanish), whereas antigens from the Spanish isolate provided protection to a challenge with the London isolate, but not against itself (Goyal & Wakelin 1993). However, so far, there is a general lack of data on the

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Table 1. Description of isofemale *S. ratti* lines used.

isofemale line	maintenance[a]	derivation
ED5	homogonic	Philadelphia, USA (Wilson & Simpson 1981)
ED43	homogonic	Edinburgh, UK (Viney *et al.* 1992)
ED132	homogonic	Kagoshima, Japan (Noda *et al.* 1987; Viney *et al.* 1992)
ED248	heterogonic	derived from heterogonic progeny of ED132
ED321	heterogonic	derived from heterogonic progeny of ED5
ED391	homogonic	Wiltshire, UK (Viney *et al.* 1992)
ED399	homogonic	Sussex, UK (Fisher & Viney 1998)
ED410	homogonic	derived from heterogonic progeny of ED132

[a] Lines are maintained by passage in Wistar rats with iL3s derived subject to heterogonic or homogonic selection (Viney 1996), i.e. with and without sexual reproduction, respectively.

effects of genotype-specific immune responses on nematode infections. Thus, host immune responses are known to reduce the *per capita* survivorship and fecundity of parasitic nematode infections in a density-dependent manner, i.e. the effect of the immune response on these traits increases with the dose that a host receives (Paterson & Viney 2002). Therefore, if acquired immune responses directed against nematode genotype A have less effect against genotype B, one would predict that *per capita* survivorship and/or fecundity would be higher in infections containing a mixture of parasite genotypes than in infections containing only genotype A. The relative lack of available evidence to support or refute this prediction is an important omission because such interactions between parasite genotypes would provide an evolutionary mechanism to maintain genetic diversity at antigen coding loci.

Interactions between nematodes of different genotype may also affect the degree of parasite aggregation, as it is not unreasonable to hypothesize that hosts may vary in various immune parameters that in turn cause variation between hosts in the magnitude of genotype-specific interactions. Previous epidemiological work stresses the importance of understanding the proximal causes of aggregation so as to understand the processes regulating parasite population dynamics (Anderson & May 1978) and so link individual-level infection processes with the population-level consequences of these processes.

Here, we use a laboratory model to address the functional consequences of parasite genetic diversity for life-history variation and for genotype-specific interactions in nematode infections.

2. MATERIAL AND METHODS

(a) *Study system*

Strongyloides ratti is a parasitic nematode that naturally infects rats (Dawkins 1989). Parasitic stages are female only and produce eggs by mitotic parthenogenesis (Viney 1994), which are passed in the faeces and can then develop by one of two developmental routes (Viney 1996; Harvey *et al.* 2000). In homogonic development, eggs develop directly into infective third-stage larvae (iL3s). In heterogonic development, eggs develop into free-living males and females, which mate and produce eggs that develop into iL3s. All parasite lines used were generated from a single iL3 and thus are known as isofemale lines (Viney 1996; Tindall & Wilson 1988) and were maintained by serial passage in Wistar rats. The isofemale lines used, their derivation and method of maintenance (Viney *et al.* 1992) are shown in table 1. Previous work has shown that the unusual life cycle of *S. ratti* has no apparent effect on the genetic structure of *S. ratti* populations, i.e. parasite genotypes are found in Hardy–Weinberg proportions and low levels of genetic differentiation are found between sites in the UK (Fisher & Viney 1998). All infections were established by sub-cutaneous inoculation of iL3s. Food and water were available *ad libitum*. Faeces were collected overnight and cultured as described previously (Viney *et al.* 1992).

The total reproductive output of an infection was determined by counting the number of worms present in cultures of faeces (Paterson & Viney 2002). The number of adult worms in a host was determined directly by observation of the gut of sacrificed animals from which food had been previously withdrawn for 16 h (Gemmill *et al.* 1997; Paterson & Viney 2002). Taken together, these measures allow us to calculate the life-history traits 'SURVIVORSHIP' and 'FECUNDITY'. The average SURVIVORSHIP of an adult worm to time t is the number of adult worms in the gut at time t divided by the inoculating dose; the average *per capita* FECUNDITY of an adult worm at time t is the total reproductive output divided by the number of parasitic females in the rat at time t.

(b) *Variability between lines in infection dynamics*

Five groups each of 16 Lewis rats (Harlan) were infected with 25 iL3s of ED5, ED43, ED391, ED399 or ED410 (table 1). All of these lines are maintained, and develop predominantly by the homogonic route. A dose of 25 iL3s was chosen because this is, approximately, the average infection intensity of *S. ratti* in the wild (Fisher & Viney 1998). A total of 80 rats were used in four blocks, each containing 20 rats. These blocks were separated in time to maintain the number of animals used at any given time at a manageable level. Rats were faecally sampled and sacrificed to determine SURVIVORSHIP and FECUNDITY as follows: (i) in blocks one and two, all rats were sampled and sacrificed at day 23 post-infection (p.i); (ii) in block three, 10 rats were sampled and sacrificed at day 6 p.i. and 10 rats at day 23 p.i; and (iii) in block four, 10 rats were sampled and sacrificed at day 6 p.i. and 10 rats at day 13 p.i.

SURVIVORSHIP and FECUNDITY were analysed by using generalized linear modelling (GLM) assuming a negative binomial error distribution with dispersion parameter k (Wilson & Grenfell 1997). SURVIVORSHIP was analysed with the number of parasitic females as the response variable and with the natural log of the inoculating dose as the offset variable to provide a *per capita* measure of survivorship. This form of GLM analysis is analogous to standard k-factor analysis (Begon *et al.*

1996), because 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). Significance of explanatory variables and their interactions were determined by using deletion testing, with the 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 total reproductive output of an infection as the response variable and the number of parasitic females as the offset variable to give a *per capita* measure.

(c) *Interactions in single-infected, mixed-line infections*

(i) *High dose*

Three groups of six Wistar rats (Harlan) were infected with either 1000 iL3s of a single line (two groups) or a mixture of 500 iL3s of each of two lines (one group). A dose of 1000 iL3s was chosen because (i) this reduces sampling noise in total reproductive output and (ii) we speculated that the effect of genotype interactions may be greater in high-dose infections. This is based on the observation that the effect of a host immune response on parasitic nematodes increases with the inoculating dose (Paterson & Viney 2002). The following pairs of lines were used; ED43 and ED132; ED43 and ED248; ED43 and ED321; ED132 and ED321; ED248 and ED321. Rats were faecally sampled on days 5, 6, 9, 13, 16 and 20 p.i. to determine total reproductive output.

(ii) *Low dose*

Three groups of eight PVG rats (Harlan) were inoculated with either (i) six iL3s of ED43, (ii) six iL3s of ED321 or (iii) a mixture of three iL3s of ED43 and three iL3s of ED248. A total dose of six iL3s was chosen to test the generality of interactions found at high doses. Rats were faecally sampled on days 5, 6, 9, 13, 16, 20, 23, 27, 30, 34 and 37 p.i. to determine total reproductive output.

(d) *Analysis of single-infected, mixed-line infections*

Reproductive output (for both high and low dose infections) was analysed in a mixed model for repeated measures (Crowder & Hand 1990; Paterson 2001). The main advantage of using a mixed model to analyse data for which there are repeat measures on individual animals is that it overcomes the problem of pseudoreplication, i.e. the correlation within a set of measures made on the same individual. Its main disadvantage is that methods are not readily available for data with a negative binomial distribution so that reproductive output is most readily analysed by using normally distributed errors after a log transformation of the response variable. Previous work has shown this to be a satisfactory model of experimental *S. ratti* infections (Paterson 2001).

The reproductive output, y, for infection with line A is given as

$$\ln \boldsymbol{y}_i = \boldsymbol{X}_{\mathrm{A}i}\boldsymbol{\beta}_{\mathrm{A}} + b_i + \boldsymbol{e}_i, \qquad (2.1)$$

where y_i is a vector of p observations $y_i = (y_1,\ldots,y_j,\ldots,y_p)^{\mathrm{T}}$ and $\boldsymbol{X}_{\mathrm{A}i}\boldsymbol{\beta}_{\mathrm{A}}$ is the fixed component, i.e. the expected value of y_{ij} independent of the individual being measured, b_i is the random component, i.e. the deviation of the expected value of $\boldsymbol{y}_i$ from $\boldsymbol{X}_{\mathrm{A}i}\boldsymbol{\beta}_{\mathrm{A}}$ specific to the individual being measured, with $b_i \sim N(0, \sigma_1^2)$ and $\boldsymbol{e}_i$ is the associated error component (Crowder & Hand 1990), with $e_{ij} \sim N(0,\sigma_e^2)$. For infections of a mixture of lines A and B, the fixed component in equation (2.1) takes the general form

$$\boldsymbol{X}_{\mathrm{M}i}\boldsymbol{\beta}_{\mathrm{M}} = \ln[\exp(\boldsymbol{X}_{\mathrm{A}i}\boldsymbol{\beta}_{\mathrm{A}}) + \exp(\boldsymbol{X}_{\mathrm{B}i}\boldsymbol{\beta}_{\mathrm{B}})] + \gamma, \qquad (2.2)$$

where γ is the effect of interaction between lines on reproductive output and is equal to zero where there is no interaction between lines. Thus, the fixed component defined by equation (2.2) is derived from the fixed terms for lines A and B in equation (2.1). Unfortunately, because of the log transformation on the response variable y, the parameters within the fixed term do not linearize and parameter estimation can only be achieved through Gibb's sampling. The infection of each line was modelled as a quadratic equation with terms for intercept, time and time squared. Parameters were estimated using Gibb's sampling in the package BUGS (http://www.mrc-bsu.cam.ac.uk/bugs). Code specific to this analysis is available from the authors on request.

The effect of interactions between lines on the level of aggregation at both high and low doses was analysed using separate GLMs assuming a negative binomial error distribution with dispersion parameter k. Separate models were fitted to each time point. At each time point, different means were fitted to each group and models were constructed that fitted either a separate k for each group or one common k to all groups (Shaw & Dobson 1996; Harvey *et al.* 1999). The difference in $2 \times$ log likelihood between these two sets of models is approximately distributed as a χ^2 with two degrees of freedom, which allows the significance of differences in aggregation between groups to be tested.

(e) *Interactions in trickle-infected, mixed-line infections*

To extend these experiments to a more natural infection process we used a trickle infection regime. Five groups of eight rats per group were infected with a total of 30 iL3s over the course of three weeks with two homogonic lines as shown in figure 1. This infection regime was used to simulate a natural infection process to give a total dose consistent with infections found in the wild (Fisher & Viney 1998). These infections were achieved either by inoculation with a single line, by inoculation with one line followed by inoculation with a second line or by inoculation with a mixture of two lines. A total of 40 rats were infected in two equal blocks. These blocks were separated in time to maintain the number of animals used at any given time at a manageable level.

For these trickle-infections the total reproductive output will derive from the combination of up to three serial inoculations. Thus, equation (2.2) becomes

$$\boldsymbol{X}_{\mathrm{M}i}\boldsymbol{\beta}_{\mathrm{M}} = \ln\left[\sum_k^3 \exp(\boldsymbol{X}_{\mathrm{A}ik}\boldsymbol{\beta}_{\mathrm{A}k}) + \sum_k^3 \exp(\boldsymbol{X}_{\mathrm{B}ik}\boldsymbol{\beta}_{\mathrm{B}k})\right] + \gamma, \qquad (2.3)$$

where k refers to the kth out of three serial infections. Parameter estimation was achieved through Gibb's sampling, as above. The effect of interactions between lines on aggregation was analysed

	time p.i. →							
	day 0	day 5	day 7	day 8	day 12	day 14	day 15	day 19
group	inoculated		inoculated			inoculated		
a	10 ED43	faecally sampled	10 ED43	faecally sampled	faecally sampled	10 ED43	faecally sampled	faecally sampled
b	10 ED43		10 ED132			10 ED132		
c	5 ED43 + 5 ED132		5 ED43 + 5 ED132			5 ED43 + 5 ED132		
d	10 ED132		10 ED43			10 ED43		
e	10 ED132		10 ED132			10 ED132		

Figure 1. Trickle infection regime. Rats were inoculated with iL3s and faecally sampled on the days shown.

Table 2. Generalized linear models of SURVIVORSHIP and FECUNDITY.

	coefficient (± s.e.m.)	change in deviance	d.f.	*p*
SURVIVORSHIP				
intercept	−0.684 ± 0.114			
block two	−0.533 ± 0.131			
block three	0.390 ± 0.110			
block four	0.123 ± 0.115	49.196	3	< 0.001
ED43	−0.185 ± 0.129			
ED391	0.065 ± 0.124			
ED399	−0.390 ± 0.135			
ED410	−0.050 ± 0.126	14.809	4	< 0.01
FECUNDITY				
intercept	0.799 ± 0.127			
block two	−0.096 ± 0.133			
block three	4.507 ± 0.157			
block four	3.283 ± 0.194	0.806[a]	3	n.s.
ED43	0.691 ± 0.149			
ED391	0.475 ± 0.149			
ED399	0.568 ± 0.149			
ED410	0.306 ± 0.149	24.941	4	< 0.001
time	0.137 ± 0.006	19.481[a]	1	< 0.001
block three · time	−0.203 ± 0.011			
block four · time	−0.094 ± 0.024	14.903	2	< 0.01

[a] These deviance values were generated by deleting the term (block or time) from a model not containing a block · time interaction term as these terms are both marginal to that interaction, i.e. the interaction term cannot be defined without the lower-order terms. *p* values for block and parasite line effects are given for the deletion of all blocks or all lines from the model. The coefficients of the line terms, ED43, ED391, ED399 and ED410, are expressed as contrasts with ED5, which acts as a baseline.

in the same manner as for the single-infection, mixed-line infections, as described above.

3. RESULTS

(a) *Variability between lines in infection dynamics*

We tested the hypothesis that there are significant differences in the life-history traits of different *S. ratti* lines. The results are shown in table 2. We found no detectable effect of time on SURVIVORSHIP, i.e. we could not detect the putative loss of parasitic females from the host gut over time. In previous work (Paterson & Viney 2002) using different infection regimes and a range of doses we have readily detected and quantified this. The failure to detect it here may be due to the low level of infection (25 iL3s inoculated) used, as the *per capita* reduction in SURVIVORSHIP increases with inoculating dose. Additionally, of the 80 rats used in this design, 50 were sampled at day 23 p.i., which may exacerbate problems estimating an effect of time on SURVIVORSHIP given that sampling points were distributed unevenly over the time-course of the infection. Nevertheless, there were significant differences between the lines in their SURVIVORSHIP averaged over the course of an infection (table 2; figure 2*a*). We also found significant differences between the lines in their FECUNDITY (table 2), as shown in figure 2*b*. In addition, we detected an effect of time on FECUNDITY, although the direction of this effect varied between blocks and so its biological interpretation or relevance is unclear. No interaction between parasite line

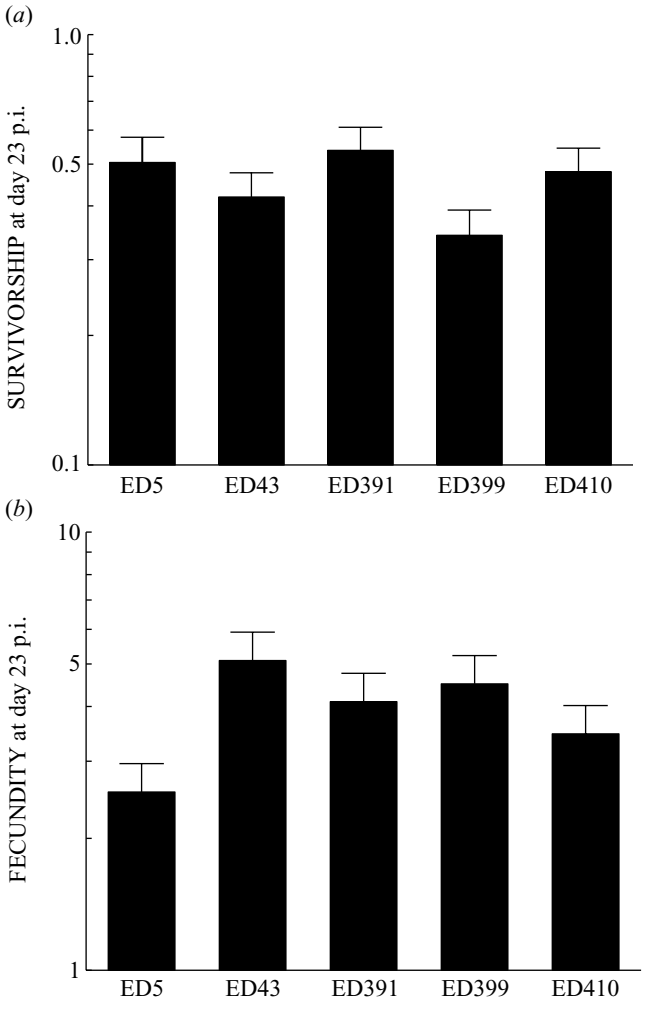

Figure 2. Differences in life-history traits between parasite lines. (*a*) SURVIVORSHIP and (*b*) FECUNDITY are shown for ED5, ED43, ED391, ED399 and ED410 using predictions and standard errors from the GLM analysis (table 2). For illustrative purposes SURVIVORSHIP and FECUNDITY are shown for block one, in which all rats were sacrificed at day 23 p.i. The same pattern of variation between lines in SURVIVORSHIP and FECUNDITY is observed at all other time points in all blocks.

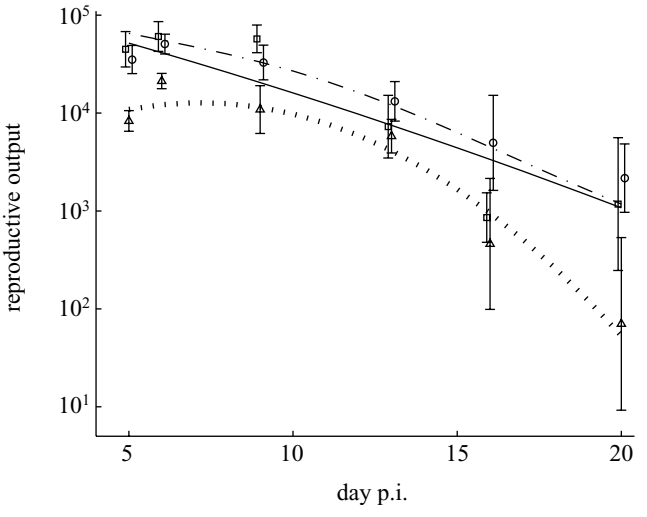

Figure 3. Single-infected, mixed-line infection of ED43 and ED248 (1000 iL3s). The reproductive output of ED43 (squares), ED248 (triangles) and mixed ED43/ED248 (circles) infections ± 1 s.e.m. (based on the variance within each group at each time point), with the predicted output (equation (2.2) and table 4) as solid line, dotted line, and dot and dashed line, respectively.

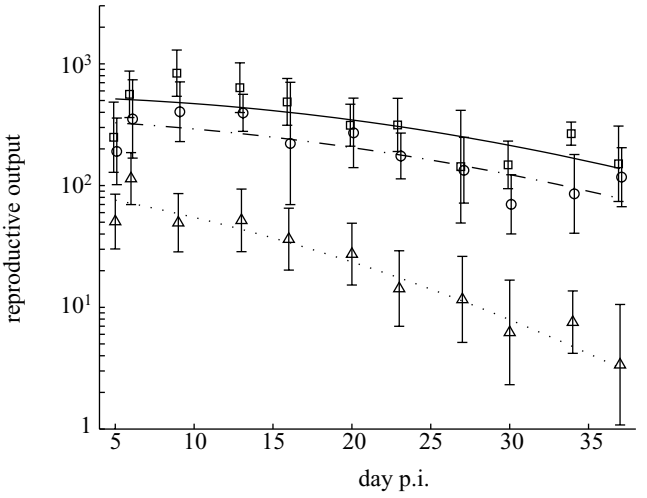

Figure 4. Single-infected, mixed-line infection of ED43 and ED248 (six iL3s). The reproductive output of ED43 (squares), ED248 (triangles) and mixed ED43/ED248 (circles) infections ± 1 s.e.m. (based on the variance within each group at each time point), with the predicted output (equation (2.2) and table 5) as solid line, dotted line, and dot and dashed line, respectively.

and time was observed for either SURVIVORSHIP or FECUNDITY, i.e. there were no detectable differences between lines in the rate of change of these traits with time. Significant block effects were found for both SURVIVORSHIP and FECUNDITY, but no interactions between parasite line and block were observed for either SURVIVORSHIP or FECUNDITY. Thus, in summary, among five isofemale lines of *S. ratti* we found significant differences in the life-history traits of survivorship and fecundity.

(b) *Interactions in single-infected, mixed-line infections*

We tested the hypothesis that different lines of *S. ratti* interact in mixed infections, such that the reproductive output of mixed infections is greater compared with single-line infections. In single-infected, mixed-line infections we tested five pairwise combinations of lines at a total inoculating dose of 1000 iL3s. Out of these five combinations just one, that of ED43 and ED248, was significantly different (table 3) compared with single ED43 or ED248 infections (table 4; figure 3). Table 4 shows that the term for interaction between lines, γ, is significantly greater than zero, leading to the rejection of the null hypothesis of no interaction between lines in mixed infections. Further analysis of this finding shows that there were only 23 out of 10 000 Gibb's samples with a value of $\gamma < 0$ (i.e. a significance level of $p \approx 0.0023$), which remains significant after Bonferoni correction for five comparisons.

To assess the generality of this interaction between ED43 and ED248, we investigated the same mixed infection but at a lower total inoculating dose of six iL3s. With

Table 3. Summary of single-infected, mixed-line infections (1000 iL3s).

	interaction (γ)[a]		
	2.5%	median	97.5%
ED43 versus ED132	−0.488	−0.156	0.194
ED43 versus ED248	0.223	0.738	1.218
ED43 versus ED321	−0.004	0.465	0.939
ED132 versus ED321	−0.899	−0.354	0.168
ED248 versus ED321	−0.643	−0.172	0.300

[a] Interactions between pairs of lines in mixed line infections were estimated from equation (2.2) using Gibb's sampling and the interaction term, γ, shown (as median and 95% confidence limits). The null hypothesis of no interaction between lines is $\gamma = 0$.

Table 4. Analysis of ED43 and ED248 single-infected, mixed-line infections (1000 iL3s).
(The reproductive output of each infection was fitted to equation (2.2) as a quadratic expansion $\alpha + \beta t + \beta' t^2$ for ED43 and ED248, where t is time in days p.i.; γ is the term for interactions between lines, and σ_b and σ_e are the standard errors of random variation between animals and the residual variation, respectively. Parameter estimates shown as median and 95% confidence limits. The null hypothesis of no interaction between lines is $\gamma = 0$.)

	2.5%	median	97.5%
α_{ED43}	10.800	11.900	13.120
β_{ED43}	−0.434	−0.188	−0.015
β'_{ED43}	−0.010	−0.003	0.007
α_{ED248}	6.361	7.725	9.116
β_{ED248}	0.233	0.478	0.709
β'_{ED248}	−0.043	−0.033	−0.023
γ	0.223	0.738	1.218
σ_b	0.822	0.951	1.101
σ_e	0.031	0.191	0.572

Table 5. Analysis of ED43 and ED248 single-infected, mixed-line infections (six iL3s).
(The reproductive output of each infection was fitted to equation (2.2) as a quadratic expansion $\alpha + \beta t + \beta' t^2$ for ED43 and ED248, where t is time in days p.i.; γ is the term for interactions between lines, and σ_b and σ_e are the standard errors of random variation between animals and the residual variation, respectively. Parameter estimates shown as median and 95% confidence limits. The null hypothesis of no interaction between lines is $\gamma = 0$.)

	2.5%	median	97.5%
α_{ED43}	5.832	6.288	6.773
β_{ED43}	−0.050	−0.004	0.037
β'_{ED43}	−0.002	−0.001	0.000
α_{ED248}	4.016	4.593	5.167
β_{ED248}	−0.108	−0.047	0.018
β'_{ED248}	−0.003	−0.001	0.000
γ	−0.272	0.105	0.474
σ_b	0.209	0.327	0.495
σ_e	0.591	0.645	0.709

this infection regime, we did not detect any significant difference in the total reproductive output of single or mixed infections (table 5; figure 4), i.e. the null hypothesis of $\gamma = 0$ was not rejected.

The results from these high and low inoculating dose experiments also show differences in the dynamics of the single-line infections, which supports and extends the findings presented in table 2 and figure 2. Thus, table 4 shows that the intercept terms for ED43 and ED248 (α_{ED43} and α_{ED248}, respectively) at high inoculating dose are significantly different from each other and that the mean reproductive output of ED248 decreases more rapidly over time (modelled by the terms β_{ED248} and β'_{ED248}) than that of ED43 (as shown in figure 3). The same pattern is observed at a low inoculating dose (table 5; figure 4). These differences between lines appear to be correlated with development such that lines that develop predominantly by the heterogonic route (ED248 and ED321) have lower initial reproductive output and a more rapid decline in reproductive output than lines that develop predominantly by the homogonic route (ED5, ED43 and ED132 'data not shown').

(c) *Interactions in trickle-infected, mixed-line infections*

We also investigated the hypothesis that different lines of *S. ratti* interact in mixed infections when those infections were established using a trickle-infection regime. However, with the lines and infection regime that we used, we did not detect any difference in the reproductive output of single- and mixed-line infections (table 6). The statistical analyses of these trickle infections require more complicated models compared with those for single-dose infections. In the analyses of the trickle infections we note that the parameter estimates of α, β and β' (coefficients of a quadratic equation defining the dynamics of the reproductive output with time) are poor for the second and third infections in the series of trickle infections, as shown by the large confidence intervals for these parameter estimates. However, the confidence intervals for all the other parameter estimates, including γ (the interaction between lines), are consistent with those found for single-dose infections (tables 4 and 5).

(d) *Analysis of aggregation in mixed-line infections*

We tested the hypothesis that parasite aggregation is greater in mixed-line infections compared with single-line infections. We did not observe any difference in aggregation between single- and mixed-line infections. For example, the results for mixed ED43 and ED248 infections at an inoculating dose of 1000 iL3s are shown in table 7 and figure 5*a*. After accounting for the different

Table 6. Analysis ED43 and ED132 trickle-infected, mixed-line infections.
(The reproductive output of infections inoculated in the kth of three serial infections was fitted as a quadratic expansion, $\alpha + \beta t + \beta' t^2$, where t is days p.i. of the kth infection. Thus, the observed reproductive output from the population of parasitic nematodes within a rat for a mixed-line infection is the combination of the separate reproductive outputs for each parasitic nematode line within each separate serial infection (equation (2.3)). γ is the term for interactions between lines, block is the overall difference between the first and second blocks, σ_b is the standard deviation of between-individual variation and σ_e is the standard deviation of residual variation. Parameter estimates shown as median and 95% confidence limits. The null hypothesis of no interaction between lines is $\gamma = 0$.)

term	2.5%	median	97.5%
first infection			
α_{ED43}	3.087	3.650	4.198
β_{ED43}	−0.019	0.122	0.241
β'_{ED43}	−0.016	−0.007	0.005
α_{ED132}	4.062	4.662	5.250
β_{ED132}	0.080	0.201	0.321
β'_{ED132}	−0.024	−0.015	−0.005
second infection			
α_{ED43}	−68.160	−12.280	3.979
β_{ED43}	−66.660	−6.052	53.430
β'_{ED43}	−71.840	−21.160	5.080
α_{ED132}	−69.290	−15.160	4.101
β_{ED132}	−67.330	−5.408	52.590
β'_{ED132}	−70.880	−20.460	5.556
third infection			
α_{ED43}	−69.910	−19.950	1.082
α_{ED132}	−66.060	−11.340	4.337
γ	−0.124	0.189	0.507
block	0.588	1.175	1.765
σ_b	0.690	0.890	1.168
σ_e	0.518	0.578	0.653

mean reproductive outputs between the three infection groups the inclusion of separate aggregation parameters (k) for each group was not supported statistically; i.e. the addition of group-specific aggregation parameters did not significantly reduce the residual deviance (table 7). In common with other studies (Pacala & Dobson 1988), there is an increase in aggregation with time in all groups at these relatively high inoculating doses ($p < 0.01$, Mantel test; table 7).

In mixed infections at low (six iL3s) inoculating doses the mean reproductive output of the three groups differed considerably during the infection (figure 4), but there was no difference in the level of aggregation in the groups (figure 5*b*; table 8). There was a significant difference between the three groups in k at just one time point (day 34 p.i.), but this was not significant after Bonferoni correction for 11 comparisons. In contrast to the high-dose infections, there was no change in aggregation during time in any group. For trickle-infected mixed-line infections, maximum-likelihood estimates of k failed to converge after 20 iterations of the fitting procedure and so an analysis of aggregation was not possible for these infections.

4. DISCUSSION

Here, we have examined a range of *S. ratti* lines for variations in life-history traits that underlie the dynamics of nematode infection. We find that lines of *S. ratti* do vary in their survivorship and fecundity (as defined by their *per capita* probability of surviving to time t and their *per capita* production of larvae at time t, respectively). These traits are crucial to the persistence of infection within hosts and to transmission between hosts. Such analyses have not been explicitly applied to parasitic nematodes and only to a limited extent in free-living nematodes. With the free-living nematode *Caenorhabditis elegans*, a comparison of wild isolates found some variation in total lifetime reproductive output, growth rate and longevity (Hodgkin & Doniach 1997). The organism that has been most intensively studied for life-history traits is *Drosophila*. Many, but not all of the studies on *Drosophila* show a trade-off between traits such as early and late reproduction, reproduction and longevity and longevity and development rate (Rose & Charlesworth 1981; Rose 1984; Partridge & Sibly 1991; Stearns 1992; Roff 2002). Analogous trade-offs in parasitic nematodes are likely to provide intrinsic constraints on their reproduction and population dynamics and on the incidence and severity of nematode disease in human and animal populations.

Do our results provide any evidence of life-history trade-offs in *S. ratti*? One potential trade-off is between survivorship and fecundity. We find that lines with higher survivorship tend to have lower fecundity at day 23 p.i. (ED5, ED391 and ED410) and, conversely, those with lower survivorship tend to have higher fecundity (ED43 and ED399) (figure 2). However, this intriguing observation is not statistically significant. The relatively low number of lines in this comparison makes the demonstration of any such significant trade-off unlikely. Thus, further work will have to be undertaken to substantiate or refute this possibility. Alternative approaches to investigating this could include artificial selection experiments. We speculate that other trade-offs may also occur; for example, between the number of eggs and the quality of these eggs (Lack 1947, 1948). This may be an important trade-off for parasitic nematodes in general and for *S. ratti* in particular. A major feature of *Strongyloides* biology is the existence of alternative development (direct, homogonic development and indirect, heterogonic development). Previous work has shown that there is substantial heritable variation in lines in the probability of larvae developing by either route (Viney 1996). Here, we find that the total reproductive output of lines that develop predominantly by the indirect, heterogonic route (ED248 and ED321) declines during an infection more rapidly than that for lines that develop by the homogonic route of development (ED43 and ED132; figures 3 and 4). Consequently, the total number of eggs produced over the course of an infection in those lines that develop predominantly by the heterogonic route is fewer than that for lines that develop predominantly by the homogonic route. This may indicate that homogonic lines invest greater resources in total into egg production than heterogonic lines. However, this observation is also consistent with a situation in which parasitic females, with finite resources available to be invested into eggs, invest greater resources into eggs des-

Table 7. Analysis of aggregation in ED43 and ED248 single-infected, mixed-line infections (1000 iL3s).
(The likelihood difference of models with a different k or a common k has an approximately χ^2 distribution with two degrees of freedom.)

day p.i.	5	6	9	13	16	20
common k[a]	11.48 ± 3.78	16.84 ± 5.56	6.37 ± 2.07	4.27 ± 1.37	1.38 ± 0.42	0.71 ± 0.20
k ED43[b]	7.64 ± 4.32	9.37 ± 5.32	10.75 ± 6.11	2.49 ± 1.35	4.37 ± 2.45	0.76 ± 0.38
k ED248[b]	20.96 ± 12.04	37.01 ± 21.31	4.22 ± 2.34	8.91 ± 5.06	0.78 ± 0.39	0.43 ± 0.20
k mixture[b]	12.20 ± 6.95	22.81 ± 13.08	7.20 ± 4.07	5.50 ± 3.08	1.69 ± 0.90	2.00 ± 1.08
likelihood difference	1.52	3.02	1.34	2.70	4.84	4.20
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[a] Overdispersion parameter, k, estimated from GLMs with different means and common k to all groups.
[b] Overdispersion parameter, k, estimated from GLMs with different means and different k in each group.

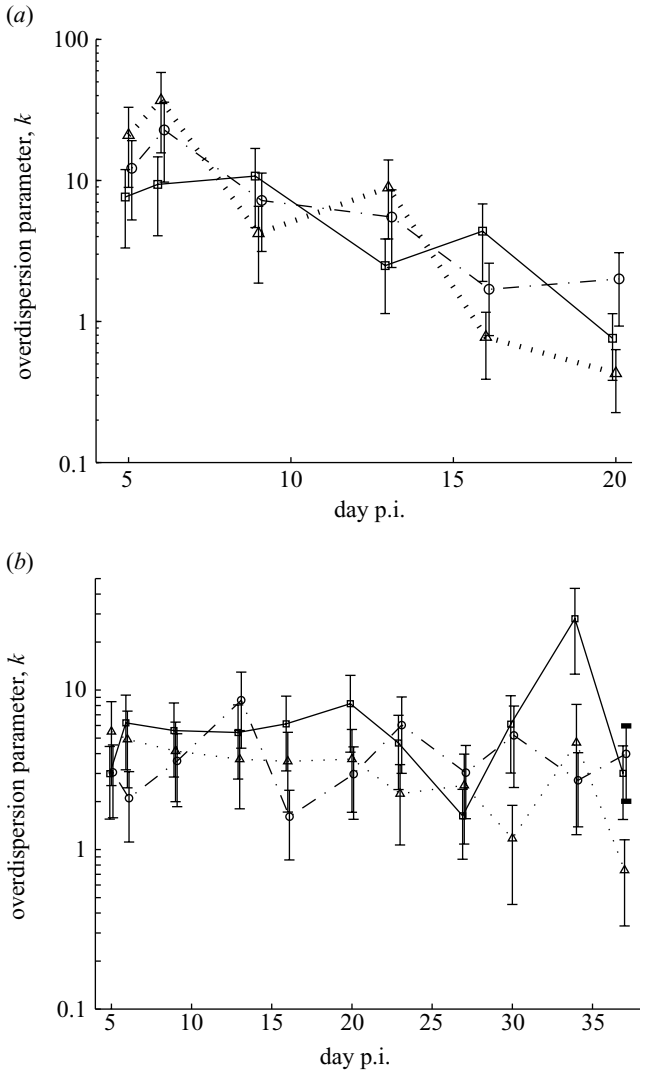

Figure 5. Aggregation in single-infected, mixed line infections. Overdispersion parameter, k, of ED43 (solid line and squares), ED248 (dotted line and triangles) and mixed ED43/ED248 (dot and dashed line and circles) infections ± 1 s.e.m. for (*a*) 1000 iL3s (table 7) and (*b*) six iL3s (table 8).

tined to develop into free-living adults than into eggs destined to develop directly into iL3s. The extra cost may be justified if investment into free-living adults is recouped by their production of additional iL3s.

We have also used different lines of *S. ratti* to examine the effect of mixed-line infections, under different infection scenarios, on the reproductive output of infections. Here, we have found some, but limited, evidence of an effect of mixed-line infections on the reproductive output of infection. Mixed-line infections are likely to be a common feature of nematode infection. Indeed, given the overdispersion of natural nematode infections, the majority of a parasite population is likely to be in mixed-line infections (Fisher & Viney 1998). Further, a major, adaptable component of the environment of parasitic nematodes is the immune response of their hosts. It is therefore not unreasonable to imagine that there are effects of mixed-line infections, possibly mediated by the host immune response, on the dynamics of nematode infection.

The parameter space in which to investigate mixed-line infections is huge. In the area that we looked we found only one case, a single, high-dose infection of ED43 and ED248, where the mixed-line infection had a greater reproductive output compared with single-line infections. In other pairwise, mixed-line infections in single- or trickle-infected infections we did not observe differences between mixed- and single-line infections. In addition, we did not find any evidence of differences in levels of parasite aggregation in mixed- or single-line infections. The infection regimes and combinations of parasite lines used in this study are not exhaustive and we cannot exclude the possibility that other combinations of lines in other infection regimes do interact. However, our findings so far would suggest that even if these interactions exist they are unlikely to be a sufficiently widespread phenomenon to be a major feature of *Strongyloides* biology. These results need to be extended to other species of parasitic nematodes to determine whether interactions between different parasite lines play any part in the epidemiology or evolution of parasitic nematodes.

Understanding life-history traits, particularly survivorship and fecundity, in parasitic nematodes has a direct relevance to understanding the epidemiology of nematode infections and controlling the diseases that they cause. Thus, the persistence of an infection is directly related to the ability of a nematode to survive within a host and the transmission of infection is directly related to nematode fecundity. By extension, any chemotherapeutic, vaccination or public health strategy that limits nematode sur-

Table 8. Analysis of aggregation in ED43 and ED248 single-infected, mixed-line infections (six iL3s).

day p.i.	5	6	9	13	16	20
common k[a]	3.50 ± 0.99	3.52 ± 0.98	4.30 ± 1.23	5.35 ± 1.57	2.78 ± 0.79	4.13 ± 1.21
K ED43[b]	2.99 ± 1.44	6.23 ± 3.07	5.58 ± 2.72	5.42 ± 2.65	6.14 ± 3.03	8.22 ± 4.14
K ED321[b]	5.49 ± 2.97	4.91 ± 2.47	4.15 ± 2.15	3.69 ± 1.88	3.58 ± 1.86	3.69 ± 1.98
k mixture[b]	3.06 ± 1.47	2.10 ± 0.98	3.59 ± 1.73	8.64 ± 4.32	1.61 ± 0.75	2.98 ± 1.43
likelihood difference	0.83	2.98	0.42	1.38	3.91	2.11
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
day p.i.	23	27	30	34	37	
common k[a]	3.86 ± 1.18	2.21 ± 0.63	3.64 ± 1.34	4.98 ± 1.64	2.06 ± 0.69	
k ED43[b]	4.66 ± 2.28	1.63 ± 0.76	6.12 ± 3.10	28.01 ± 15.43	3.01 ± 1.46	
k ED321[b]	2.24 ± 1.17	2.53 ± 1.44	1.17 ± 0.72	4.69 ± 3.45	0.74 ± 0.41	
k mixture[b]	6.03 ± 3.03	3.03 ± 1.47	5.20 ± 2.75	2.72 ± 1.33	3.99 ± 1.98	
likelihood difference	2.01	0.92	4.65	8.66	5.60	
p	n.s.	n.s.	n.s.	< 0.05	n.s.	

[a] Overdispersion parameter, k, estimated from GLMs with different means and common k to all groups.
[b] Overdispersion parameter, k, estimated from GLMs with different means and different k in each group.

vivorship and fecundity will limit the incidence and severity of nematode disease. A further, major advantage of analysing life-history traits explicitly is that it allows a large body of ecological theory on life-history evolution to be applied to parasitic nematodes. Various epidemiological models already use the life-history traits survivorship and fecundity to analyse the dynamics of parasite populations (Anderson & May 1992; Grenfell *et al.* 1995). However, these models are not framed explicitly within the context of life-history theory. In particular, we are not aware of epidemiological models that consider variation in life-history traits or trade-offs between life-history traits in parasitic nematodes. Further development of epidemiological models in these areas, accompanied by field and laboratory studies are required. These will provide significant advances in our understanding of the processes that have shaped the evolution of life-history traits in parasitic nematodes, the forces that maintain genetic diversity in these traits and the role of trade-offs between life-history traits to provide natural constraints on nematode infections.

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REFERENCES

Anderson, R. M. & May, R. M. 1978 Regulation and stability of host–parasite population interactions: regulatory processes. *J. Anim. Ecol.* **47**, 219–247.

Anderson, R. M. & May, R. M. 1992 *Infectious diseases of humans: dynamics and control*. Oxford University Press.

Anderson, T. J. C., Blouin, M. S. & Beech, R. N. 1998 Population biology of parasitic nematodes: applications of genetic markers. *Adv. Parasitol.* **41**, 220–283.

Begon, M., Harper, J. L. & Townsend, C. R. 1996 *Ecology*. Oxford: Blackwell.

Belosovic, M. & Dick, T. 1979 *Trichinella spiralis*: comparison of stages in host intestine with those of an Arctic *Trichinella* sp. *Exp. Parasitol.* **48**, 432–446.

Chadee, K. C. & Dick, T. 1982 Biological characteristics and host influence on a geographical isolate of *Trichinella* (wolverine: 55N 100W, 1979). *J. Parasitol.* **68**, 451–456.

Crawley, M. J. 1993 *GLIM for ecologists*. Oxford: Blackwell Scientific.

Crowder, M. J. & Hand, D. J. 1990 *Analysis of repeated measures*. London: Chapman & Hall.

Dawkins, H. J. S. 1989 *Strongyloides ratti* infections in rodents: value and limitations as a model of human strongyloidiasis. In *Strongyloidiasis: a major roundworm infection of man* (ed. D. I. Grove), pp. 287–332. London: Taylor & Francis.

Fisher, M. C. & Viney, M. E. 1998 The population genetic structure of the facultatively sexual parasitic nematode *Strongyloides ratti* in wild rats. *Proc. R. Soc. Lond.* B **265**, 703–709. (DOI 10.1098/rspb.1998.0350.)

Gemmill, A. W., Viney, M. E. & Read, A. F. 1997 Host immune status determines sexuality of a parasite nematode. *Evolution* **51**, 393–401.

Goyal, P. K. & Wakelin, D. 1993 Vaccination against *Trichinella spiralis* in mice using antigens from different isolates. *Parasitology* **107**, 311–378.

Grenfell, B. T., Wilson, K., Isham, V., Boyd, H. E. G. & Dietz, K. 1995 Modelling patterns of parasite aggregation in natural populations: trichostrongylid nematode–ruminant interactions as a case study. *Parasitology* **111**(Suppl.), 135–151.

Harvey, S. C., Paterson, S. & Viney, M. E. 1999 The distribution of *Strongyloides ratti* infective stages among the faecal pellets of rats. *Parasitology* **119**, 227–235.

Harvey, S. C., Gemmil, A. W., Read, A. F. & Viney, M. E. 2000 The control of morph development in the parasitic nematode *Strongyloides ratti*. *Proc. R. Soc. Lond.* B **267**, 2057–2063. (DOI 10.1098/rspb.2000.1249.)

Hodgkin, J. & Doniach, T. 1997 Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**, 149–164.

Lack, D. 1947 The significance of clutch size 1. Intraspecific variation. *Ibis* **89**, 302–352.

Lack, D. 1948 The significance of litter size. *J. Anim. Ecol.* **17**, 45–50.

McCullagh, P. & Nelder, J. A. 1989 *Generalised linear models. Monographs on statistics and applied probability*. London: Chapman & Hall.

Noda, S., Uchikawa, R., Mori, T. & Sato, A. 1987 A survey of *Angiostrongylus cantonesis* in the port side areas of Kagoshima city and Makurazaki city, Kagoshima prefecture. *Jpn. J. Parasitol.* **36**, 100–102.

Pacala, S. W. & Dobson, A. P. 1988 The relation between the number of parasites and host age: population causes and maximum likelihood estimation. *Parasitology* **96**, 197–210.

Partridge, L. & Sibly, R. 1991 Constraints in the evolution of life histories. *Phil. Trans. R. Soc. Lond.* B **332**, 3–13.

Paterson, S. 2001 The use of repeated measure linear modelling to analyze longitudinal data from experimental parasite infections. *J. Parasitol.* **87**, 969–971.

Paterson, S. & Viney, M. E. 2000 The interface between epidemiology and population genetics. *Parasitol. Today* **16**, 528–532.

Paterson, S. & Viney, M. E. 2002 Host immune responses are necessary for density dependence in helminth infections. *Parasitology* **125**, 283–292.

Pozio, E., la Rosa, G., Rossi, P. & Murrel, K. D. 1992 Biological characterisation of *Trichinella* isolates from various host species and geographical regions. *J. Parasitol.* **78**, 647–653.

Read, A. F. & Viney, M. E. 1996 Helminth immunogenetics: why bother? *Parasitol. Today* **12**, 337–343.

Roff, D. A. 2002 *Life history evolution*. Sunderland, MA: Sinauer.

Rose, M. R. 1984 Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38**, 1004–1010.

Rose, M. R. & Charlesworth, D. 1981 Genetics of life history in *Drosophila melanogaster*. II Exploratory selection experiments. *Genetics* **97**, 187–196.

Shaw, D. J. & Dobson, A. P. 1996 Patterns of macroparasitic abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**(Suppl.), 111–133.

Stearns, S. C. 1992 *The evolution of life histories*. Oxford University Press.

Tindall, N. R. & Wilson, P. A. G. 1988 Criteria for proof of migration routes of immature parasites inside hosts exemplified by studies of *Strongyloides ratti* in the rat. *Parasitology* **96**, 551–563.

Viney, M. E. 1994 A genetic analysis of reproduction in *Strongyloides ratti*. *Parasitology* **109**, 511–515.

Viney, M. E. 1996 Developmental switching in the parasitic nematode *Strongyloides ratti*. *Proc. R. Soc. Lond.* B **263**, 201–208.

Viney, M. E., Matthews, B. E. & Walliker, D. 1992 On the biological and biochemical nature of cloned populations of *Strongyloides ratti*. *J. Helminthol.* **66**, 45–52.

Wakelin, D. & Goyol, P. K. 1996 *Trichinella* isolates: parasite variability and host responses. *Int. J. Parasitol.* **26**, 471–481.

Wilson, K. & Grenfell, B. T. 1997 Generalized linear modelling for parasitologists. *Parasitol. Today* **13**, 33–38.

Wilson, P. A. G. & Simpson, N. A. 1981 Dynamics of infection of rats given low doses of homogonic and heterogonic *Strongyloides ratti*. *Parasitology* **83**, 459–475.

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