Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite

C. M. Davies¹*, J. P. Webster¹ and M. E. J. Woolhouse^{1,2}

¹Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

²Centre for Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, UK

The adaptive trade-off theory for the evolution and maintenance of parasite virulence requires that virulence be genetically correlated with other fitness characteristics of the parasite. Many theoretical models rely on a positive correlation between virulence and transmissibility. They assume that high parasite replication rates are associated with a high probability of transmission (and, hence, increased parasite fitness), but also with high levels of damage to the host (high virulence). Schistosomes are macroparasites with an indirect life cycle involving a mammalian and a molluscan host. Here we demonstrate, through the development of five substrains, a genetic basis for schistosome virulence. We used these substrains further in order to investigate the presence of parasite fitness traits that were genetically correlated with virulence. High virulence in the (mouse) definitive host was, as predicted, positively correlated with parasite replication. In contrast, in the (snail) intermediate host high virulence was associated with low parasite replication rates. Variation in infectivity to and parasite replication in the definitive host was suggested as a compensating mechanism for the maintenance of virulence in the snail host. This is the first report of a trade-off in parasite reproductive success across hosts in an indirectly transmitted macroparasite.

Keywords: virulence; infectivity; transmission; *Schistosoma mansoni*; parasite; *Biomphalaria glabrata*

1. INTRODUCTION

Modern evolutionary theory considers parasite virulence, i.e. the damage done to the host and the reduction in host fitness, to be a consequence of parasites adapting to natural host populations in order to maximize their own fitness (Imhoof & Schmid-Hempel 1998). So long as there is sufficient genetic variability on which to act, natural selection operates on the rate of spread of parasites between hosts and will tend to maximize the total number of successful offspring produced in hosts infected by a single parasite strain (Anderson & May 1982). Virulence reduces parasite fitness by reducing the duration of infection. However, if virulence is correlated with other factors, such as the rate of transmission, maximizing parasite fitness involves trade-offs between the production of transmission stages (which ideally should be high) and damage to the host (which ideally should be small) (May & Anderson 1990) since linkage between traits constrains their simultaneous evolution (Ebert & Herre 1996). Indeed, a positive correlation between parasite virulence and parasite replication rate and between parasite replication rate and transmission success are two fundamental assumptions of many theoretical models for the evolution of parasite virulence (Bull 1994).

However, most studies of parasite virulence have considered directly transmitted microparasites and there has been significantly less theoretical or experimental attention paid to macroparasites or indirectly transmitted parasites. Snail–schistosome interactions are a useful model system in which to investigate such evolutionary

hypotheses. Schistosomes are macroparasites with an indirect life cycle involving a mammalian and a molluscan (freshwater snail) host. Transmission between hosts occurs via free-swimming larval stages, miracidia (infective to the molluscan intermediate host) and cercariae (infective to the mammalian definitive host). Schistosomes are pathogenic parasites of both their molluscan and mammalian hosts and several studies have suggested that there may be variability in schistosome virulence (Wiest 1996; Webster & Woolhouse 1999).

A trait must have a genetic basis in order to be subject to natural selection. A powerful way of demonstrating the presence of genetic variation for a trait is through isofemale lines in which a laboratory strain is bred from a single mated individual (Parsons 1980). In this study, five substrains of *Schistosoma mansoni* were developed from the mating of single clones of female schistosomes to single male clones in order to investigate genetic variation for virulence. Genetic correlations of virulence with other fitness traits, including parasite replication rate, parasite success, transmission to definitive hosts and pathogenicity in definitive hosts, were then investigated.

2. MATERIAL AND METHODS

(a) *Host and parasite strains*

Five substrains were developed from a laboratory strain of *S. mansoni*, which was originally from Puerto Rico (Sm-PR). The parental strain had been routinely passaged through a mixed genotype pool of *Biomphalaria glabrata* and CBA/CA mice for more than 20 generations, but had been demonstrated to be genetically polymorphic by random-amplified polymorphic DNA polymerase chain reaction analysis (Williams *et al.* 1990; C. M. Davies, unpublished data). All snails were sexually mature individuals (measured by the onset of egg laying) of an

*Author for correspondence
(charlotte.davies@wellcome-epidemiology.oxford.ac.uk).

Figure 1. Process of successful development of one parasite substrain. Eight separate mice were infected in the F₀ generation and five miracidia-producing substrains were recovered.

inbred strain of *B. glabrata* (originally from Brazil) that had been maintained in the laboratory without parasite pressure for *ca.* 20 generations.

(b) Host and parasite maintenance

All snails were maintained at 23–25 °C and subjected to a light regime (full ultraviolet spectrum, SAD Lightboxes Co. Ltd, High Wycombe, UK) of 12 L:12 D. Snails were housed in individual plastic pots (10 cm × 10 cm × 30 cm) in a standard volume of a 50% deionized water:50% Caledonian spring water mix. The water was changed weekly and snails were fed *ad libitum* on freeze-dried lettuce.

(c) Selection of parasite substrains (see figure 1)

(i) F₀

One hundred and twenty *B. glabrata* were exposed to a single miracidium of the unselected parental parasite strain in *ca.* 6 ml of deionized water for 2 h, a period sufficient for maximal miracidial penetration (Lewis *et al.* 1986). There is a latency or

‘pre-patent’ period of *ca.* 35–45 d from exposure of snails to miracidia to production of cercariae. From week 4 and weekly thereafter cercariae from each snail were ‘shed’ by placing snails in the dark for 24 h and subsequently exposing them to an overhead light source (100 W) at 10.00 for 2 h in vials containing 20 ml deionized water. Patent infections are those that produce cercariae and can thus potentially infect definitive hosts. Individual snails were thus scored as positive or negative for cercarial production and 17 patently infected snails were identified. Infected snails were randomly paired and used to infect eight six-week-old inbred CBA/CA mice by paddling for 1 h in 30 ml of infected water. Each snail contributed 110 cercariae and, thus, each mouse received a dose of 220 cercariae. Since single miracidial infections had been used, cercariae arising from a single snail represented a single-sexed clonal population derived by asexual reproduction and, thus, *ca.* 50% of mice were expected to have mixed-sex infections.

Mice were killed using carbon dioxide at seven weeks post-infection before signs of pathology had occurred. Miracidia arising from a single mouse were considered a single parasite substrain. Five egg-producing substrains were recovered, arising from crosses between a male and a female cercarial clone infection, and were named M1 to M5. Livers were macerated through a sieve in 100 ml of deionized water and exposed to a bright light for 30 min in order to cause hatching of snail-infective parasite stages, i.e. miracidia. Separate sieves were used for each substrain in order to prevent cross-contamination.

(ii) F₁

Groups of 25 size-matched (9–11 mm in diameter), sexually mature *B. glabrata* were exposed to a dose of six miracidia per snail for each parasite substrain. A control group of 25 snails was not infected but subjected to identical treatment throughout. Five infected snails were randomly chosen for each substrain at ten weeks post-infection. There was no selection based on life-history traits. Cercariae shed from selected snails were pooled and used to infect four inbred six-week-old CBA/CA mice at a dose of 220 cercariae per mouse. Substrains were maintained for a further generation under identical conditions. Thus, all parasite substrains were expected to experience identical levels of inbreeding, which is a prerequisite for comparison of genetic traits across strains.

(d) Life-history data

The following parameters were measured for each parasite substrain in each generation: (i) virulence and infectivity to the snail intermediate host, (ii) parasite reproduction in snail hosts, (iii) virulence and infectivity to the mouse definitive host, and (iv) parasite reproduction in the mouse definitive host.

- (i) Individual snail survival was recorded daily. Virulence was defined as the reduction in host survival attributable to parasite infection. Reduction in host survival is the most appropriate measure of virulence for an obligatorily horizontally transmitted parasite, particularly one transmitted to a long-lived definitive host, since parasite success is not influenced by the number of host offspring produced. A parasite substrain was considered to be less virulent than another substrain if hosts exposed to the former had a relatively higher survival. Snails were shed weekly in 20 ml of deionized water as described above. Infectivity was recorded as a binary variable, with patent infections awarded a score of 1.

Table 1. *Parasite virulence and reproduction in the intermediate host of five substrains of S. mansoni*

(Back-transformed means and standard errors of the means for substrains M1–M5 are shown, adjusted for snail size and generation. Degrees of freedom for *F*-values: all snails = 4,235 and patent snails = 4,151. The values were square-root transformed for analysis for all snails for virulence to the snail host, the parasite replication rate and parasite success in the host snail and for patent snails for parasite success in the host snail. The values were log transformed for patent snails for virulence to the host snail.)

	M1	M2	M3	M4	M5	<i>F</i>	<i>p</i>
infectivity to the host snail (frequency of patent infection)							
all snails	0.631 ± 0.048	0.848 ± 0.048	0.870 ± 0.091	0.567 ± 0.063	0.416 ± 0.063	9.14	0.026
virulence to the host snail (host survival/control survival)							
all snails	0.461 ± 0.001	0.743 ± 0.001	0.667 ± 0.005	0.588 ± 0.003	0.468 ± 0.003	3.88	0.005
patent snails	0.463 ± 0.087	0.687 ± 0.074	0.633 ± 0.132	0.545 ± 1.122	0.448 ± 0.155	5.00	0.001
parasite replication rate (number of cercariae produced by day 46)							
all snails	445.2 ± 5.62	918.1 ± 5.76	1183.4 ± 18.7	228.0 ± 11.6	98.0 ± 13.1	14.79	< 0.001
patent snails	1265.5 ± 160.1	1715.6 ± 139.4	1900.8 ± 240.1	1041.4 ± 222.6	873.5 ± 278.0	4.71	0.001
intensity of cercarial production (mean weekly number of cercariae)							
all snails	1083.1 ± 161.9	1781.1 ± 163.7	2011.8 ± 295.2	1053.8 ± 232.9	561.0 ± 247.2	7.29	< 0.001
patent snails	1690.0 ± 202.4	2257.0 ± 176.2	2499.0 ± 303.5	1879.0 ± 281.4	1167.0 ± 351.4	4.19	0.003
length of pre-patent period (number of weeks)							
patent snails	5.28 ± 0.10	5.37 ± 0.09	5.26 ± 0.17	5.17 ± 0.16	5.66 ± 0.19	1.23	0.325
parasite success in the host snail (total number of cercariae produced)							
all snails	6068.4 ± 130.0	19 126.9 ± 134.6	21 785.8 ± 432.6	6256.8 ± 269.0	1616.0 ± 306.3	9.14	< 0.001
patent snails	12 432.3 ± 198.8	28 561.0 ± 148.8	30 660.0 ± 445.2	19 126.9 ± 380.3	7276.1 ± 595.4	5.16	0.001

- (ii) The intensity of cercarial shedding in patently infected snails was estimated weekly from the number of cercariae in ten 0.2 ml samples per snail (after ensuring a homogeneous distribution of cercariae in each vial by gentle shaking), which were stained with iodine and assayed under the light microscope. The number of parasite stages produced at an early stage in an infection can be considered to be a measure of the parasite replication rate (Taylor *et al.* 1998). We used the number of cercariae produced at day 46, which is a composite measure determined by the frequency of patent infections, the length of the pre-patent period and the intensity of cercarial shedding. Day 46 was chosen since this was the median length of the pre-patent period exhibited in these infections and, thus, was considered the most sensible measure for reflecting the contribution of all three of these factors, which may be considered components of the parasite replication rate. Collection of cercarial data weekly throughout the study further allowed estimation of the mean weekly cercarial production averaged throughout the experiment and an index of overall parasite success or fitness in the snail host, which was measured as the total production of cercarial stages during the experiment. The maximum survival of a patently infected snail was 46 weeks. Preliminary studies determined that there were no chronobiological differences in the emergence of cercariae between the parasite substrains (C. M. Davies, unpublished data).
- (iii) Definitive host infectivity was calculated as the number of worms recovered divided by the cercarial dose (220). Adult schistosomes were recovered by a modified hepatic perfusion technique (Smithers & Terry 1965). Worms were sexed and counted. The major pathological effect of *S. mansoni* in definitive hosts results from granuloma formation around eggs excreted from paired adults resulting in

hepatosplenomegaly (Wiest 1996). Thus, virulence to infected mice was recorded as the weight of the liver and spleen as a percentage of body weight. It was not considered ethical to assay virulence as mortality in mice and all mice were killed before visible distress occurred.

- (iv) The number of miracidia present in the liver was estimated from the mean number of miracidia in ten 0.5 ml samples. The number of miracidia produced is a measure of parasite replication rate and/or reproductive success in the mammalian host.

(e) *Data analysis*

Statistical analyses were performed using the SPSS statistical package (SPSS, Inc., Chicago, IL, USA). The presence of genetic variation in the Sm-PR schistosome strain for virulence and other fitness traits was investigated using analysis of variance (in a general linear modelling procedure). Variations in these parameters due to factors other than parasite genetic substrain were expected to be randomly distributed amongst experimental groups. Parasite substrain, parasite generation and snail size were used as independent factors. All variables were transformed as necessary in order to meet the model assumptions of normality of error and homogeneity of variance, and mean and standard errors were back-transformed for presentation. Infectivity to snail hosts was investigated using logistic regression. Since individual animals were used to investigate multiple fitness traits, Bonferroni-corrected *p*-values of *p* = 0.005 (intermediate host data) and *p* = 0.01 (definitive host data) were considered evidence of significant genetic variation between parasite substrains. Genetic trade-offs between pairs of parasite fitness traits were investigated by comparing the mean values per parasite substrain using Pearson correlation coefficients. It should be noted that, in these experiments, since we were looking for genetic correlations, replicates are parasite substrains (Kraaijeveld & Godfray 1997) and, hence, the statistical power

Table 2. *Parasite virulence and reproduction in the definitive host of five substrains of S. mansoni*

(Back-transformed means and standard errors of the means for substrains M1–M5 are shown, adjusted for generation. $n=40$. The values were square-root transformed for liver weight divided by body weight and spleen weight divided by body weight for virulence to the mouse definitive host, infectivity to the mouse definitive host and parasite success in the mouse definitive host. The values were log transformed for sex ratio of adult worms. Degrees of freedom for F -values = 4,33.)

	M1	M2	M3	M4	M5	F	p
virulence to mouse definitive host							
liver weight/body weight	0.123 ± 0.00006	0.106 ± 0.00006	0.078 ± 0.0003	0.129 ± 0.0001	0.119 ± 0.0003	6.23	0.001
spleen weight/body weight	0.019 ± 0.00005	0.015 ± 0.00005	0.009 ± 0.0002	0.023 ± 0.0001	0.021 ± 0.0002	4.84	0.003
infectivity to mouse definitive host (number of worms recovered)							
	35.8 ± 0.34	18.4 ± 0.33	16.1 ± 1.42	78.0 ± 0.71	48.9 ± 1.41	6.69	< 0.001
sex ratio of adult worms (number of female worms/number of male worms)							
	1.64 ± 0.057	1.79 ± 0.055	0.98 ± 0.116	1.05 ± 0.081	1.03 ± 0.116	6.50	< 0.001
parasite success in mouse definitive host (number of miracidia produced)							
	1656.5 ± 4.49	670.8 ± 4.32	681.2 ± 18.6	1239.0 ± 9.24	1772.4 ± 18.6	8.66	< 0.001

of the tests was relatively low (since $n=5$). However, each substrain represented data from multiple individuals and generations.

3. RESULTS

There was a significant cost of infection in the snail host. Snails exposed to parasites had a significantly reduced survival compared to control snails that were not exposed but otherwise treated identically ($F_{1,256}=13.12$ and $p<0.001$). Furthermore, there was a significant reduction in survival within experimental groups in snails that shed cercariae compared to those that were exposed but never shed cercariae ($F_{1,200}=6.83$ and $p=0.01$).

Table 1 shows the differences in fitness traits between the five parasite substrains in snail hosts. Although not significant, there was an indication of a difference between experimental groups in the infectivity of the parasite substrains. Infectivity (measured as the number of patent infections) was highest in substrains M3 and M2 and lowest in substrains M5 and M4. Since whether or not snails become patently infected strongly affects the number of cercariae produced and the survival of host snails (Webster & Woolhouse 1999), the results for other measures are presented for both the whole data set and for the subset of patently infected snails. There was a significant difference between the parasite substrains in virulence to the snail host: virulence was high (i.e. snail survival low) in substrains M1, M4 and M5 but significantly lower in substrains M2 and M3. This was seen in the whole data set, despite the higher frequency of patent infections in substrains M2 and M3, and more strongly in the patent subset. There was no evidence of a difference in survival between the groups in snails that were exposed but never shed cercariae ($F_{1,40}=1.77$ and $p=0.81$). The parasite replication rate differed significantly between the substrains, being highest in substrains M2 and M3. This was not solely a reflection of the higher frequency of patent infections in these substrains, since the same pattern was also seen in the patent subset. The length of the pre-patent period did not differ between the substrains and, thus, the difference in the parasite replication rate was due to the higher

intensity of cercarial shedding throughout the experiment in substrains M2 and M3. Parasite success, as measured by the total number of cercariae recorded, also differed significantly between the parasite substrains, being substantially higher in substrains M2 and M3.

The survival of snail hosts differed significantly between the F_1 and F_2 generations ($p=0.001$ for all snails and $p<0.001$ for patently infected snails) but the interaction terms (substrain $\times$ generation) were not significant, indicating that the relative characteristics of parasite substrains were stable across generations. No other fitness trait differed significantly between the two generations.

Table 2 shows the differences between the parasite substrains in definitive hosts. Infectivity to mouse definitive hosts differed between the parasite substrains. Despite all mice being exposed to identical cercarial doses, approximately fourfold fewer worms were recovered per mouse in substrains M2 and M3 than in substrains M4 and M5. There was also a difference in the sex ratio of adult worms recovered, with substrains M1 and M2 being female dominated. Hepatosplenomegaly was high in substrains M1, M4 and M5 and low in substrains M2 and M3. Parasite reproductive success, as measured by the number of miracidia produced in definitive hosts, also differed significantly between the substrains, being substantially lower in substrains M2 and M3. There were no significant interactions between parasite substrain and generation, confirming that virulence and reproductive characteristics were stable across generations.

Correlations between the means of the fitness traits are presented in table 3. Despite the relatively low power of these tests, there was evidence of strong positive correlations between infectivity to snail hosts and survival of ($r=0.88$) and parasite replication in ($r=0.98$) patently infected snails. Similarly, total parasite success in snail hosts was positively correlated with parasite infectivity ($r=0.99$), overall host survival ($r=0.88$) and the parasite replication rate in patently infected snails ($r=0.98$). Measures of infectivity, virulence and parasite replication were positively correlated in definitive hosts ($r=0.74$ – 0.99). However, there was no evidence of a relationship between these measures and

Table 3. Pearson correlation coefficients across substrains of mean fitness traits of five substrains of *S. mansoni*

(Fitness traits are shown for all snails (all), the subset of patently infected snails (patent) and mouse definitive hosts. Significant correlations are highlighted: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.)

	SSP	SCA	SCP	SI	SPA	SPP	SRA	SRP	MLV	MSV	MI	MSR	MP
snail survival (all) (SSA)	0.99**	0.85*	0.88*	0.83*	0.88*	0.93*	0.76	0.78	-0.58	-0.62	-0.53	0.14	-0.97**
snail survival (patent) (SSP)	—	0.90*	0.92*	0.88*	0.93*	0.96**	0.82	0.83*	-0.63	-0.68	-0.59	0.17	-0.99***
intensity of cercarial shedding (all) (SCA)	—	—	0.97**	0.99***	1.00***	0.96**	0.98**	0.98**	-0.81*	-0.88*	-0.78	0.17	-0.94**
intensity of cercarial shedding (patent) (SCP)	—	—	—	0.95**	0.97**	0.99***	0.92*	0.91*	-0.74	-0.80	-0.62	0.07	-0.95**
infectivity to host snail (% patent infections) (SI)	—	—	—	—	0.99***	0.93*	0.99***	0.98**	-0.76	-0.85*	-0.81*	0.30	-0.92*
parasite success in host snail (all) (SPA)	—	—	—	—	—	0.97**	0.98**	0.98**	-0.79	-0.86*	-0.77	0.20	-0.96**
parasite success in host snail (patent) (SPP)	—	—	—	—	—	—	0.92*	0.91*	-0.69	-0.75	-0.58	0.10	-0.97**
parasite replication rate in host snail (all) (SRA)	—	—	—	—	—	—	—	1.00***	-0.82*	-0.90*	-0.86*	0.26	-0.88*
parasite replication rate in host snail (patent) (SRP)	—	—	—	—	—	—	—	—	-0.86*	-0.92*	-0.88*	0.22	-0.89*
virulence to mouse host (liver weight) (MLV)	—	—	—	—	—	—	—	—	—	0.99**	0.81*	0.24	0.74
virulence to mouse host (spleen weight) (MSV)	—	—	—	—	—	—	—	—	—	—	0.87*	0.09	0.78
infectivity to mouse host (number of adult worms) (MI)	—	—	—	—	—	—	—	—	—	—	—	-0.34	0.66
sex ratio of adult worms (female:male) (MSR)	—	—	—	—	—	—	—	—	—	—	—	—	-0.09
parasite success in mouse host (number of miracidia) (MP)	—	—	—	—	—	—	—	—	—	—	—	—	—

Figure 2. Negative genetic correlation of parasite success in the snail intermediate host and mouse definitive host across five substrains of *S. mansoni*. Mean values per substrain of total cercarial production for all snails and of the total number of miracidia produced are shown.

the sex ratio of the worms recovered. Conversely, there were strong inverse relationships between fitness traits in the snail and mouse hosts; infectivity to and parasite success in the snail was negatively correlated with infectivity and success in the definitive host ($r = 0.81$ and $r = -0.96$, respectively). The strong inverse relationship of parasite success in the intermediate and definitive hosts is shown in figure 2.

4. DISCUSSION

This study has demonstrated significant variations in virulence to both intermediate and definitive hosts that are attributable to infecting schistosome genotype. Thus, we have demonstrated a genetic basis for virulence and, further, that virulence polymorphisms are maintained even within an inbred laboratory strain.

The two assumptions of the adaptive trade-off theory for the evolution of parasite virulence are a positive genetic relationship between (i) the parasite replication rate and virulence, and (ii) parasite replication and transmission (Bull 1994; Mackinnon & Read 1999). Virulence in the mouse definitive hosts was associated with the number of worms establishing and their reproductive success and is thus consistent with the assumption that high virulence is a by-product of high parasite replication. However, variations in the infectivity of miracidia suggested that the number of miracidia produced was not linearly related to transmission success. Consideration of the order of magnitude of increased miracidial production (being approximately fourfold higher in the high virulence substrains) compared to the reduced infectivity of miracidia produced (being approximately twofold lower in these high virulence substrains) does nevertheless suggest that high parasite replication rates will be associated with increased transmission.

However, an opposite situation occurred in the snail hosts where increasing parasite replication rate was associated with decreasing rather than increasing

virulence. Moreover, although there was a reduction in host survival associated with patent infection, virulence was lowest in the parasite substrains that produced the most patent infections. Further, the snail–schistosome model did not support the assumption of increased transmission associated with an increased parasite replication rate. In fact, parasite replication in the snail host was strongly negatively correlated with infectivity to and parasite reproductive success in the mouse definitive host. Overall, there were negative correlations of infectivity, parasite replication and total parasite reproductive success in the intermediate and definitive hosts. This is, to the authors' knowledge, the first report of trade-offs of this nature across hosts in an indirectly transmitted parasite. Thus, virulence to the snail host may have been maintained in schistosome populations by a positive relationship with transmission to the definitive host, but neither virulence nor transmission success were correlated with the parasite replication rate.

One potential explanation for such a negative correlation between parasite reproductive success in definitive and intermediate hosts may be that of 'antagonistic pleiotropy'. Organisms have a finite pool of resources that they can allocate to different functions and, thus, these trade-offs may reflect differential investment in the two hosts of the life cycle, either energetically or in physiological adaptation to the widely differing environments of the molluscan and mammalian hosts (Frank 1996; Fellowes *et al.* 1999). Another possibility is that a single gene or gene complex with multiple alleles has an influence on infectivity to and/or reproductive rates in both intermediate and definitive hosts or that adjacent loci are involved in linkage disequilibrium (Kraaijeveld & Godfray 1997). However, the issue as to why faster-replicating parasites should be less virulent in the snail intermediate host than slowly replicating ones is more complex. High rates of parasite replication presumably result in increased usage of host tissues and nutritional resources, as well as increased damage to tissues and the presence of high levels of parasite antigens or metabolic by-products (Anderson & May 1982). High virulence may be the result of a specific tissue tropism (Levin & Svanborg Eden 1990). An alternative explanation is that virulence may be a result of poor compatibility between host and parasite. Compatibility is measured as the ability of the schistosomes to infect intermediate host snails successfully and high compatibility in both laboratory and field populations is characterized by a high frequency of patent infections, high levels of cercarial production and low host mortality (Barbosa 1975; Ward *et al.* 1988). Although much still needs to be understood about the precise mechanisms involved, it appears that, in compatible interactions, the snail does not launch an effective defensive response and parasites successfully establish and develop (Richards *et al.* 1992). Both host defensive ability and parasite immuno-evasive mechanisms are thought to be important in determining whether a defence response occurs (Rollinson & Southgate 1985). If such a response is costly to the host, either energetically or through immunopathological damage, high compatibility may thereby be characterized by high parasite replication rates but also with reduced virulence, as observed in this study. This explanation is supported by histological examinations of *B. glabrata*–*S. mansoni* infections. Extensive

tissue reactions and disturbances in blood flow due to the formation of emboli (blood vessel obstructions) consisting of degenerating cercariae have been observed in moderately compatible infections (Pan 1965). In contrast, in more compatible host–parasite strain combinations such cercarial emboli have not been observed and extensive tissue reactions do not appear until later in the course of an infection (Schiff 1975).

A trade-off in reproduction and fitness between snail and definitive hosts is potentially highly significant for the epidemiology and transmission of schistosomiasis in natural foci, since the production of eggs is the major cause of morbidity in human infection (Wiest 1996) and the existence of such trade-offs may be one reason why transmission control has proved particularly difficult in schistosomiasis control. Genetic trade-offs are also an important component in the maintenance of polymorphisms in traits in natural populations (Frank 1993).

In summary, parasite genetic variation for infectivity and virulence to and replication rate in the snail intermediate and mouse definitive hosts was demonstrated in a laboratory *S. mansoni* strain. Virulence was positively genetically correlated with transmission success, suggesting that virulence will not evolve to zero because of the fitness benefits to parasites in terms of transmission that are associated with high virulence. However, neither virulence nor transmission success in the snail intermediate host were positively correlated with the parasite replication rate as is commonly assumed in mathematical models of the evolution of parasite virulence. Instead, there was a trade-off in parasite reproductive success in the intermediate and definitive hosts, the first report of a trade-off of this nature in any host–parasite system. We therefore suggest that indirectly transmitted parasites would benefit from specific consideration in theoretical models of parasite virulence evolution.

We thank Mrs Karen Clifton and Ms Viv Clarke for assistance with animal husbandry and Professor David Goldstein for helpful discussion. This research was funded by the EPA Cephalosporin Trust and the Medical Research Council. Animal procedures were conducted under the Animals (Scientific Procedures) Act 1986 administered by the Home Office (project licence 30/02747).

REFERENCES

- Anderson, R. M. & May, R. M. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411–426.
- Barbosa, F. S. 1975 Survival and cercaria production of Brazilian *Biomphalaria glabrata* and *B. straminea* infected with *Schistosoma mansoni*. *J. Parasitol.* **61**, 151–152.
- Bull, J. J. 1994 Perspective: virulence. *Evolution* **48**, 1423–1437.
- Ebert, D. & Herre, E. A. 1996 The evolution of parasitic diseases. *Parasitol. Today* **12**, 96–101.
- Fellowes, M. D. E., Kraaijeveld, A. R. & Godfray, H. C. J. 1999 Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Evolution* **53**, 1302–1305.
- Frank, S. A. 1993 Specificity versus detectable polymorphism in host–parasite genetics. *Proc. R. Soc. Lond. B* **254**, 191–197.
- Frank, S. A. 1996 Models of parasite virulence. *Q. Rev. Biol.* **71**, 37–78.
- Imhoof, B. & Schmid-Hempel, P. 1998 Single-clone and mixed-clone infections versus host environment in *Crithidia bombi* infecting bumblebees. *Parasitology* **117**, 331–336.

- Kraaijeveld, A. R. & Godfray, H. C. J. 1997 Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**, 278–280.
- Levin, B. R. & Svanborg Eden, C. 1990 Selection and the evolution of virulence in bacteria: an ecumenical excursion and modest suggestion. *Parasitology* **100**, S103–S115.
- Lewis, F. A., Stirewalt, M. A., Souza, C. P. & Gazzinelli, G. 1986 Large-scale laboratory maintenance of *Schistosoma mansoni*, with observations of three schistosome/snail host combinations. *J. Parasitol.* **72**, 813–828.
- Mackinnon, M. J. & Read, A. F. 1999 Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* **53**, 689–703.
- May, R. M. & Anderson, R. M. 1990 Parasite–host coevolution. *Parasitology* **100**, S89–S101.
- Pan, C. 1965 Studies on the host–parasite relationship between *Schistosoma mansoni* and the snail *Australorbis glabratus*. *Am. J. Trop. Med. Hyg.* **14**, 931–976.
- Parsons, P. A. 1980 Isofemale strains and evolutionary strategies in natural populations. In *Evolutionary biology* (ed. M. Hecht, W. Steere & B. Wallace), pp. 201–235. New York: Plenum Publishing Corporation.
- Richards, C. S., Knight, M. & Lewis, F. A. 1992 Genetics of *Biomphalaria glabrata* and its effects on the outcome of *Schistosoma mansoni* infection. *Parasitol. Today* **8**, 170–174.
- Rollinson, D. & Southgate, V. R. 1985 Schistosome and snail populations: genetic variability and parasite transmission. In *Ecology and genetics of host–parasite interactions* (ed. D. Rollinson & R. M. Anderson), pp. 91–109. London: Academic Press.
- Schiff, C. H. J. 1975 Studies on the South African strain of *S. mansoni*. III. Notes on certain host–parasite relationships between intra-molluscan larvae and intermediate hosts. *S. Afr. J. Sci.* **71**, 8–20.
- Smithers, S. R. & Terry, R. J. 1965 The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology* **55**, 695–700.
- Taylor, L. H., Mackinnon, M. J. & Read, A. F. 1998 Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*. *Evolution* **52**, 583–591.
- Ward, R. D., Lewis, F. A., Yoshino, T. P. & Dunn, T. S. 1988 *Schistosoma mansoni*: relationship between cercarial production levels and snail host susceptibility. *Exp. Parasitol.* **66**, 78–85.
- Webster, J. P. & Woolhouse, M. E. J. 1999 Cost of resistance: relationship between reduced fertility and increased resistance in a snail–schistosome host–parasite system. *Proc. R. Soc. Lond. B* **266**, 391–396.
- Wiest, P. M. 1996 The epidemiology of morbidity of schistosomiasis. *Parasitol. Today* **12**, 215–220.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* **18**, 6531–6535.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.