Cost of resistance: relationship between reduced fertility and increased resistance in a snail–schistosome host–parasite system

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Natural host populations often exhibit genetic variability in resistance to parasitism. One possible mechanism for maintaining such diversity is a trade-off between fitness costs associated with resistance and fitness costs associated with parasitism. However, little is known about the nature or magnitude of these costs in animal populations. Using artificial selection experiments in a *Biomphalaria glabrata*–*Schistosoma mansoni* host–parasite system, we demonstrated that resistance and susceptibility to infection are heritable. We then investigated whether resistance had any associated costs in terms of snail reproductive success. Susceptible-selected snail lines showed significantly higher fertility (number of offspring produced) than resistant-selected or unselected control snail lines, irrespective of current infection status. There were no consistent differences between snail lines in fecundity, proportion of abnormal egg masses produced, or mean number of eggs per egg mass. Mortality rate was higher among infected than uninfected snails. These results are consistent with snails incurring costs of resistance to schistosome infection in the absence of the parasite.

Keywords: costs of resistance; coevolution; fertility; fecundity; *Biomphalaria glabrata*; *Schistosoma mansoni*

1. INTRODUCTION

Natural host populations often exhibit considerable genetic variability in resistance to parasitism. Coevolutionary theory suggests that one potential mechanism for maintaining such diversity is a trade-off between fitness costs associated with resistance and fitness costs associated with parasitism (May & Anderson 1983; Fritz & Simms 1992; Frank 1994). However, convincing demonstrations of costs of resistance and their underlying causes have proved elusive, particularly in animal host–parasite systems (Fritz & Simms 1992).

Snail–schistosome interactions are a useful model system in which to investigate such evolutionary hypotheses. Schistosomes, the causative agents of schistosomiasis, are macroparasites with an indirect life cycle involving a mammalian and a molluscan host. Transmission between hosts occurs via free-swimming larval stages, miracidia (infective to the mollusc) and cercariae (infective to the mammal). The susceptibility of snails to schistosome infection is known to vary both between and within snail species (Richards 1970, 1975; Basch 1975, 1976; Manning *et al.* 1996; Morand *et al.* 1996; Webster & Woolhouse 1998). At least some of this within-species variation is heritable (Richards 1970; Webster & Woolhouse 1998) but, clearly, resistance does not become fixed in field populations (Woolhouse 1989a).

The aim of this study was to investigate potential costs of resistance in a *Biomphalaria glabrata*–*Schistosoma mansoni* host–parasite system. A powerful technique for identifying trade-offs between fitness components is the study of correlated responses to artificial selection (Kraaijeveld & Godfrey 1997). We thus used artificial selection to breed snails that were resistant or susceptible to schistosome infection, and investigated whether this had any associated cost in terms of snail fertility (measured as actual reproductive performance; using the number of offspring produced) and/or fecundity (measured as potential reproductive capacity; using the number of eggs and embryos formed).

2. MATERIALS AND METHODS

Snails were maintained in the laboratory at 23–26 °C, and subjected to a light (full UV spectrum; SAD lightboxes Co. Ltd) 12 L:12 D regime (lights on between 08.00 and 20.00). Snails were kept in plastic tanks containing UV and de-copper treated, aerated tap water. The tanks were changed weekly, and snails were fed *ad libitum* on freeze-dried lettuce and fish food (Tetra). All snail tanks contained Styrofoam sheets, on which the snails would preferentially deposit their eggs.

Laboratory strains used were of the normally susceptible *B. glabrata* snails, and *S. mansoni* parasites. Snail and parasite lines had been maintained independently in the laboratory for many (each > 20) generations, but had been demonstrated to be genetically polymorphic by randomly amplified-polymorphic-DNA PCR analysis (Webster & Woolhouse 1998). As part of work described elsewhere (Webster & Woolhouse 1998), artificial

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Figure 1. Snail lines and artificial selection methods. Flow diagram shown for first replicate of selected and control lines only (for clarity). Arrows represent division of snail tanks into uninfected (resistant-selected) and infected (susceptible-selected) subgroups (P₁s and F₄s)—see text for further details. Within each generation, all snail tank replicates were maintained at a matched breeding population size to prevent differential inbreeding effects. Artificial selection was continued in the F₄–F₅ generations from all but one replicate snail line (not shown). Likewise, subgroups of 15 snails were removed from each snail line at each generation to test for any unselected change in snail compatibility or pre-patent period (not shown—see text for further details). Asterisk denotes exposure of F₄ resistant-selected snails resulted in too low an infection rate ($n = 2$) for statistical analysis.

selection was carried out on all four combinations of two snail and two parasite strains. As there was no effect of snail–parasite combination on any of the parameters of interest here, only pooled results are presented.

(a) Artificial selection

Artificially selected snail lines either resistant or susceptible to *S. mansoni* were then established (figure 1). Six large (51 cm × 31 cm) tanks were set up, each containing 60 sexually mature snails (as indicated by egg production), matched for age and size. In each of four tanks, all the snails were individually exposed to five *S. mansoni* miracidia. A ratio of five miracidia

per snail was chosen as this is known to result in an infection rate of *ca.* 50% of the snail population, thereby helping to prevent initial differential inbreeding effects, and avoiding the high snail mortality associated with larger miracidial doses (Webster & Woolhouse 1998; Richards 1970). Snails in the remaining two tanks served as controls and were not exposed to any parasite (although otherwise treated identically).

Between four and seven weeks after parasite exposure, all snails (including the controls) were screened weekly (i.e. each snail was screened four times) by keeping the snails in darkness for 48 h and then exposing them, at 10.00, in vials containing 25 ml of de-chlorinated water, to a bright (100 W) overhead

light source for 2 h. All patently infected snails (i.e. those shedding cercariae) were marked with nail varnish. At week 7, all experimental tanks were subdivided into two new separate tanks, each containing either those to be ‘resistant-selected’ (not shedding at any point during weeks 4–7) or ‘susceptible-selected’ (shedding at any point during weeks 4–7) to infection. Any offspring and egg masses produced before the week 7 division were removed (although this was minimal, as early reproduction is low (Rupp 1996)). The control tanks were accordingly matched for mean snail population size. Thus there were now ten tanks, consisting of four resistant-selected snail line replicates, four susceptible-selected snail line replicates, and two unselected control snail line replicates.

P₁ parental lines were then left to breed for 15 weeks. F₅s from each experimental tank, again matched for age/size, were then placed in new (51 cm × 31 cm) tanks, after being exposed individually to five *S. mansoni* miracidia. Again, all snails were screened four to seven weeks later, and only those snails consistent with their selection group were maintained in the tanks for future breeding, i.e. any snails found to be susceptible within a resistant-selected tank or vice versa were removed. This breeding and selection protocol was continued until the F₅ generation. Within each generation, the number of breeding individuals in every tank was matched (to the minimum available) across snail selected lines and replicates, in order to prevent differential inbreeding effects.

At each (F₂–F₅) generation, two additional subsets of 15 snails were removed from each tank, exposed individually to five miracidia and then shed at weekly intervals over their lifetime. This was performed in order to detect any unselected change in snail compatibility (in control tanks) or in pre-patent period (in selected tanks, i.e. to confirm that the resistant-selected snail lines were in fact resistant rather than simply having an extended pre-patent period before shedding). Fitness data from these snail subsets were not included in the analyses.

Potential maternal effects were controlled for by setting up two groups of 14 unexposed–uninfected F₅ pairs consisting of (i) susceptible-selected snails crossed with susceptible-selected snails; and (ii) resistant-selected snails crossed with resistant-selected snails. Cross-breeding was confirmed by RAPD analysis (J. P. Webster, unpublished data). After 15 weeks, all mature F₆ snails bred from each replicate were then exposed to five miracidia and screened four to seven weeks post-exposure. If the response to selection was simply due to phenotype (i.e. that the offspring of parasitized snails were themselves more susceptible to parasitism, or that offspring of unparasitized snails were more resistant), rather than to genotype, then the infection rate of F₆ snails should be identical between groups.

(i) Experiment 1

The reproductive success from each snail tank replicate over each generation (P₁–F₃) was monitored at five-week intervals (each count was confirmed by a second observer). Five parameters were recorded: (i) the number of breeding adults remaining from the original population placed in each tank (mortality); (ii) the total number of all egg masses in each tank; (iii) the mean number of eggs per egg mass (a mean from an arbitrarily selected group of ten (normal) egg masses was calculated; eggs and embryos within each egg mass are easily visible to the naked eye); (iv) the number of malformed egg masses (small and mis-shaped egg masses containing few or no eggs were occasionally seen); (v) the number of offspring per tank.

(ii) Experiment 2

Any differences in reproductive success observed during selection must also be demonstrated in the absence of parasitism to establish that these differences are due to genotypic (resistant-selected–susceptible-selected status) rather than phenotypic (individual infection status) effects. F₄ snails from each selected line were placed in individual isolation pots (14 cm × 8 cm). Five groups of ten individual snails were set up (figure 1), measuring compatibility status in terms of cercarial production post exposure as described above for the artificial selection regime itself: group (i) consisted of susceptible-selected exposed–infected snails (S+); group (ii) of susceptible-selected unexposed–uninfected snails (S–); group (iii) of control exposed–infected snails (C+); group (iv) of control unexposed–uninfected snails (C–); and group (v) of resistant-selected unexposed–uninfected snails (R–). A sixth group of resistant-selected exposed–infected snails (R+) was set up, but was excluded from analyses as insufficient numbers ($n=2$) resulted in a patent infection, due to the high proportion of resistant snails in the F₄ generation.

The five parameters of reproductive success defined above were monitored for each individual snail at five-week intervals. Egg masses from these snails were also examined microscopically to determine embryo status within each egg, and wherever possible, actual numbers of offspring hatching from each egg mass were recorded.

(b) Statistical analyses

Statistical analyses were performed using the SAS package (SAS Institute 1989). Differences in the fraction of susceptible snails between selected lines were tested by log-linear analysis using the CATMOD procedure. Trends in susceptibility in the F₁ to F₄ generations were tested for using Kendall’s concordance test. Differences in measures of fertility and fecundity for snails from different lines (measured 15 weeks for P₁–F₃s and at 5, 10 and 15 weeks for F₄s) were tested using the GLM procedure. Here, variables were transformed where appropriate and residuals inspected for normality. Differences in the mortality of F₄ snails from different groups measured at the same times were tested using χ^2 tests.

3. RESULTS

Both resistance and susceptibility to schistosome infection were demonstrated to be heritable (figure 2). There were highly significant differences between susceptible-selected, resistant-selected and control snail lines by the F₁ generation (log-linear analysis: $\chi^2=93.83$, d.f.=2, $p<0.001$) and this difference was significant in all subsequent generations. In both susceptible- and resistant-selected lines, there was a trend for a further response to selection over the F₁ to F₄ generations, but this was not statistically significant. Unselected control snail lines remained at close to 50% susceptibility throughout ($\chi^2<1$, d.f.=4, $p>0.90$).

No snail from the resistant-selected lines started to shed for the first time after the seven-week post-infection cut-off point. This indicates that these snails were resistant rather than simply having an extended pre-patent period.

There were significant differences in susceptibility between resistant- and susceptible-selected F₆ snail lines: offspring from susceptible line F₅ crosses and resistant line F₅ crosses were 81% and 28% susceptible,

Figure 2. Effect of artificial selection on compatibility to *S. mansoni* infection—mean percentage of snails infected after exposure to five miracidia across generations P₁–F₄. Bars represent s.e.m. across replicates ($n=4$ per resistant-selected lines per generation; $n=4$ per susceptible-selected lines per generation; $n=2$ per unselected control lines per generation).

respectively ($\chi^2=107.83$, d.f.=7, $p<0.0001$) despite there being no exposure of the parents. This indicates that differences in susceptibility between snail lines could not be due to maternal effects.

(i) Experiment 1

Overall, there was a significant difference in the numbers of offspring produced (fertility) between snail lines (P₁–F₃: $F_{2,28}=19.45$, $p=0.0001$) and this changed as selection progressed ($F_{6,28}=5.61$, $p=0.007$). The emerging trend for a higher offspring production in the susceptible-selected snail lines, followed by that of the controls, with the lowest in resistant-selected snail lines, reached significance by the F₂ generation ($F_{2,7}=11.70$, $p=0.004$) and remained significant thereafter.

The number of adult snails remaining (mortality) also differed between snail lines over all generations (P₁–F₃: $F_{2,28}=23.10$, $p=0.001$) and increased as selection progressed ($F_{6,28}=11.94$, $p=0.0001$). There was a trend towards higher mortality among susceptible-selected (and hence infected) adults than among either resistant-selected or controls, which reached significance by the F₃ generation ($F_{2,7}=42.95$, $p=0.0001$).

No other measure of reproductive output showed a significant difference between the snail lines.

(ii) Experiment 2

Experimental support that the difference in fertility between the snail lines was related to selection on genotype rather than phenotype is shown in figure 3a. There were significant differences in numbers of offspring from unexposed snails between lines, with the highest numbers from susceptible-selected snails and the lowest from resis-

Figure 3. (a) Mean number of offspring produced per snail per group. Bars represent s.e.m. across replicates ($n=10$ snails per group). (b) Number of adult snails surviving (from $n=10$ snails per group). S+, infected susceptible-selected snail lines; S–, uninfected susceptible-selected snail lines; C+, infected control snail lines; C–, uninfected control snail lines; R–, uninfected resistant-selected snail lines. Measures were taken at point samples every five weeks (see text for further details).

tant-selected snails (week 15: $F_{2,27}=6.15$, $p=0.006$). Comparison of numbers of offspring in positive versus negative infection status groups showed no effect of parasitism (week 15: $F_{1,37}=0.0$, $p=0.95$).

Adult mortality was high and occurred in all snail groups (figure 3b), as is common when snails are maintained in isolation (Rupp 1996). However, snail line alone had no effect on mortality (comparing C+ versus S+, and C– versus S– versus R– at weeks 5, 10, and 15: all $\chi^2<1$, d.f.=1, $p>0.05$). In contrast, positive infection status significantly increased mortality (infected versus uninfected at weeks 5, 10 and 15: $\chi^2=2.22$, 4.69, 7.41, d.f.=1, $p=0.14$, 0.03, 0.006, respectively).

Microscopic examination of egg masses showed that very few eggs within resistant-selected snail lines contained developing embryos. Likewise, the offspring–egg mass ratio was significantly higher among the unexposed, susceptible-selected snails, followed by that of the controls and lowest in the resistant-selected snails ($F_{2,27}=5.21$, $p=0.01$), suggesting that the observed differences between snail lines were due to hatching rates rather than increased offspring mortality in resistant-selected lines.

No other (fecundity) factor showed any significant difference between snail groups.

4. DISCUSSION

Observations of numbers of offspring produced by the artificially selected lines strongly suggested lower fertility among resistant snails. These snails produced fewer offspring than susceptible lines, even though the prevalence of schistosome infection was significantly higher in the latter. The fact that this was not due to phenotypic selection was confirmed by experimental study of snails from resistant, susceptible and control lines in the absence of parasitism: unexposed resistant snails produced significantly fewer surviving offspring by 15 weeks (figure 3*a*). As mortality rates did not differ between unexposed lines (figure 3*b*), the number of offspring produced by 15 weeks is considered to be a good measure of lifetime reproductive success. These results thus suggest a cost of resistance in the absence of parasitism.

The data also illustrate a cost of parasitism, as adult mortality was lower among uninfected snails (figure 3*b*) as has been reported elsewhere (Woolhouse 1989*b*). This is considered to lead to higher lifetime reproductive success as significantly more uninfected than infected snails survive past 15 weeks (figure 3*b*) and, although the number of surviving offspring at 15 weeks does not differ between infected and uninfected snails (figure 3*a*), the surviving snails continue to lay viable eggs.

Together, these results support the hypothesis that costs of resistance may contribute to the maintenance of polymorphisms in snail susceptibility to schistosomes in the presence of the parasite. The results are unlikely to be due simply to differential effects of inbreeding, as proposed for Cooper *et al.*'s (1994) study using isolated, self-fertilized snails (Cousin *et al.* 1995), as in our study, selected and unselected snail lines were maintained at and bred from approximately equal population sizes (typically, *ca.* 60 snails). Nor does it appear that resistant snails are simply investing resources in defence rather than reproduction, as proposed by Minchella (1985), as the apparent energetic cost of reproduction (in terms of egg production and other fecundity measures) did not differ between snail lines. There appears instead to be some feature which prevents the eggs laid by resistant snails from developing into embryos and/or hatching into viable offspring. This may be a deficiency in internal factors which, although primarily associated with host reproduction, are also essential for normal parasite development. Possibilities include factors related to albumin gland function (Cousin *et al.* 1995), essential nutrients, or developmental signals for the immature parasite (Cooper *et al.* 1994). It is not known

whether a single gene or gene complex has pleiotropic effects on susceptibility and fertility, or whether adjacent loci are involved in linkage disequilibrium.

The existence of costs of resistance also raises the converse question of how polymorphisms in susceptibility are maintained in the absence of parasitism (as with these established laboratory populations of snails), given that selection against resistance is expected. Potential explanations include the existence of benefits of resistance alleles even in the absence of parasitism; a possible mechanism is frequency dependent mate choice with respect to resistance–susceptibility status (Rupp 1996; J. P. Webster, unpublished data). However, it may be that there is indeed strong selection for susceptible snails in the absence of the parasite. This may have been a feature of a recent schistosomiasis epidemic that occurred in a new focus in the Ndombo area of northern Senegal, in which the prevalence of *S. mansoni* infection in the *Biomphalaria pfeifferi* snail population was exceptionally high (Stelma *et al.* 1993). This may have reinforced another potentially important factor, namely the absence of any acquired immunity within the human population.

Selection for susceptible snails has, of course, wider implications for schistosomiasis control measures: if reduction of transmission rates favours an increase in the susceptibility of the snail population then this increases the likelihood of the re-emergence of the disease should control measures be relaxed. Again, this will reinforce any increased susceptibility of the human population due to loss of acquired immunity. Furthermore, our results are relevant to another proposed schistosomiasis control strategy, the 'genetic control technique', which sets out to interrupt the life cycle of schistosomes in the wild by increasing the proportion of genetically resistant intermediate host snails (Hubendick 1958; Richards 1970; Woodruff 1978, 1985; Fletcher 1985). One of the major premises on which the technique is based is that susceptible snails suffer a loss of reproductive fitness as compared to their resistant counterparts, and hence natural selection will increase the proportion of alleles for resistance (Woodruff 1985; Fletcher 1985). Our data suggest that this premise may not always be correct.

More generally, however, selection pressures in the field may be more complex than those occurring under laboratory conditions. In many habitats, planorbid snails have a dynamic metapopulation structure with frequent local extinctions and recolonizations (Woolhouse & Chandiwana 1989; Webster *et al.* 1996; Hoffman *et al.* 1998; Davies *et al.* 1998) and their exposure to schistosome infection is highly variable in space and time (Woolhouse & Chandiwana 1989). In these circumstances, selection for either resistance or susceptibility is likely to be extremely slow (Gillespie 1975). Moreover, costs of resistance may not be the only factor involved. An earlier study (Webster & Woolhouse 1998) demonstrated that snail susceptibility to schistosome infection may be strain specific, *i.e.* selection for resistance or susceptibility to one parasite strain does not confer resistance or susceptibility to other strains. This is consistent with another potential mechanism for maintaining polymorphisms, namely dynamic coevolution between parasite and host (Morand *et al.* 1996). Costs of resistance and dynamic coevolution are not

mutually exclusive and both may be important in the snail–schistosome system.

To conclude, this study of *B. glabrata* has demonstrated an association between increased resistance to parasitic schistosomes and reduced snail fertility. This may provide one of the few demonstrations of a cost of resistance in an animal host–parasite system, which has implications both at the theoretical and applied level.

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