Compatibility and sex in a snail–schistosome system

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SUMMARY

Knowledge of the genetics underlying resistance to parasitic infection has important repercussions for our understanding of infection dynamics and the mechanisms of host–parasite co-evolution. The aim here was to determine for a *Biomphalaria glabrata–Schistosoma mansoni* system whether (1) resistance is dominant over susceptibility, (2) it is possible to crossbreed snails to be simultaneously resistant and/or susceptible to more than one parasite strain and (3) compatibility genotype affects reproductive strategy. Using replicate snail strains artificially selected for either resistance or susceptibility to single replicate parasite strains, individual snails from each line were paired with a selected partner of matched or non-matched compatibility status and cross-breeding was identified by RAPD–PCR. The resulting compatibility phenotype of all offspring was determined. Support for all 3 hypotheses were obtained. The results are discussed in terms of their applied and theoretical implications.

Key words: compatibility, genetics, sex, schistosomes, snails, co-evolution.

INTRODUCTION

Knowledge of the genetics underlying resistance to parasitic infection has important repercussions for our understanding of the mechanisms of host–parasite coevolution (Clarke, 1976; Hamilton, 1980; Morand, Manning & Woolhouse, 1996; Dybdahl & Lively, 1998; Webster & Woolhouse, 1998, 1999), and allows empirical evaluation of genetic models such as that of the 'gene-for-gene' (Flor, 1956; Frank, 1992) or 'matching allele at multiple loci' models (Frank, 1996), and of co-evolutionary theories such as the Red Queen hypothesis (Hamilton, 1980; Hamilton, Axelrod & Tanese, 1990) or the maintenance of sex (e.g. Hamilton, 1980; Hamilton *et al*. 1990; Howard & Lively, 1994). Unfortunately, however, despite the large body of both theoretical and empirical plant–pathogen work available, relatively few animal host–parasite co-evolution studies have yet been performed.

Snail–schistosome interactions constitute a useful system in which to test such models and hypotheses. Although the precise nature of the genes and associated products responsible remain unknown, variations in the ability of schistosomes to successfully infect intermediate host snails (i.e. compatibility) is known to vary between snail species, populations and strains (Newton, 1952; Richards, 1975*a*, *b*; Richards & Shade, 1987; Richards, Knight & Lewis, 1992; Webster & Woolhouse, 1998), and reciprocal cross-infection experiments suggest that parasites may be adapted to their local host population (Manning, Woolhouse & Ndamba, 1995; Webster & Woolhouse, 1998; Morand *et al*. 1996; Lively & Dybdahl, 2000). Moreover, recent research has demonstrated that *B. glabrata–S. mansoni* compatibility is heritable and specific to single co-selected strains of parasite (Webster & Woolhouse, 1998, 1999).

The aim of this study was to elucidate further the genetics underlying compatibility for a *B. glabrata–Schistosoma mansoni* system by determining (1) whether resistance is dominant over susceptibility (as is common for many plant–pathogen (Fritz & Simms, 1992) and other animal-helminth (Behnke *et al*. 2000; Richards, 1975*a*, *b*) interactions), (2) whether it is possible to cross-breed snails to be resistant and/or susceptible to more than one parasite strain (this novel study contrasts to the single-strain specificity demonstrated by Webster & Woolhouse (1998) and would provide further support for the 'matching alleles at multiple loci' genetic model) and finally (3) what implications this may have on the host mating system utilized (to the author's knowledge, this is the first investigation of the potential impact of host compatibility genotype on reproductive strategy in this system, and could have implications for coevolutionary theories regarding both the genetic underpinnings of compatibility and the maintenance of sex (Hamilton, 1980; Hamilton *et al*. 1990; Howard & Lively, 1994)).

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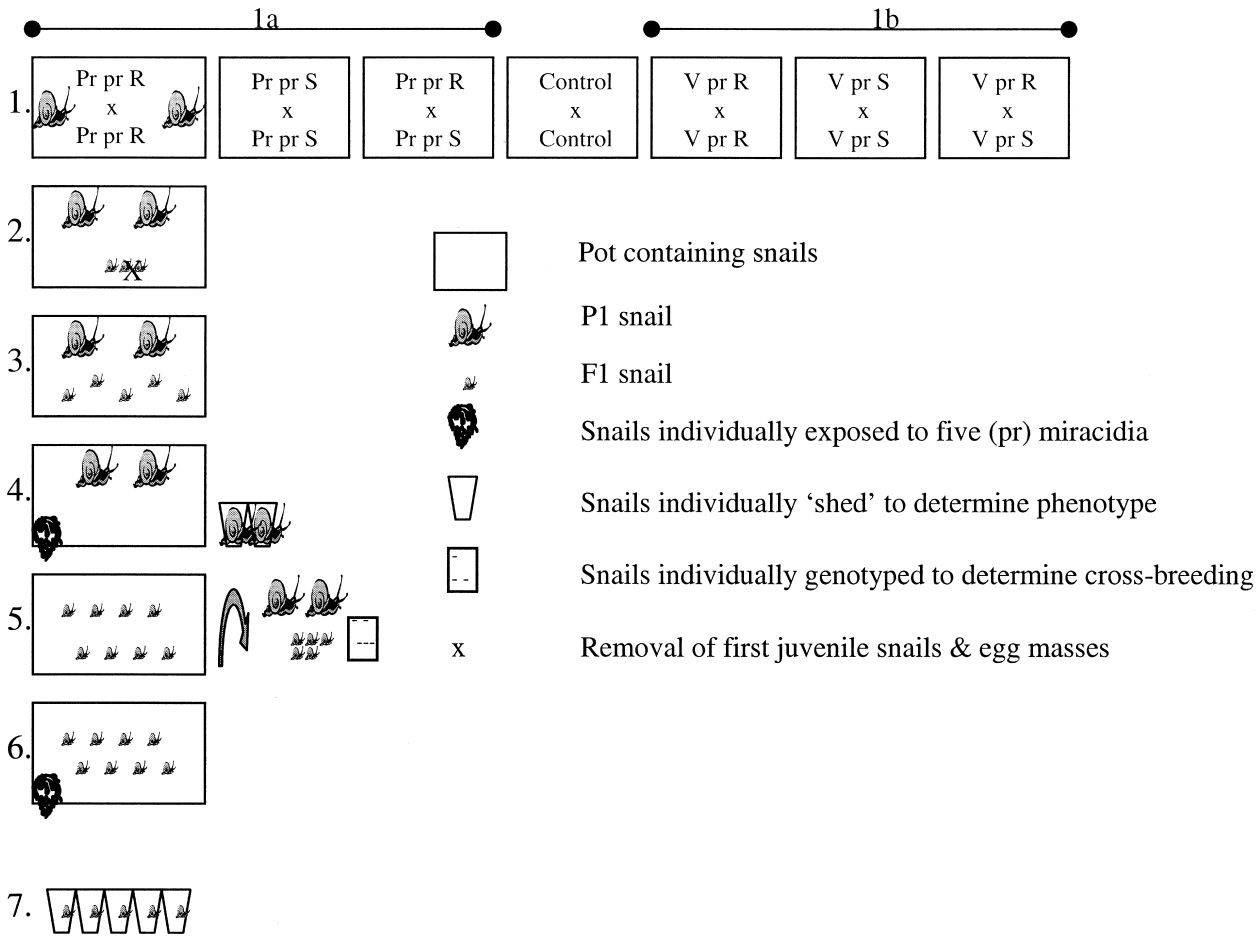

Fig. 1. Methodology used to test for Dominance of Resistance (Exp. 1a and b). Details shown for first group (of $n \leqslant 14$ replicates) only for clarity. PR, Puerto Rican snail strain; V, Vespiano snail strain; Control, unselected snail strain; pr, Puerto Rican parasite strain; R, resistant-selected snail lines; S, susceptible-selected snail line. P1 and F1 snails tested at matched age/size, although illustrated in Fig. 1 as different sizes for clarity only. The numbered steps 1–7 refer directly to those detailed in the Materials and Methods section in the text.

MATERIALS AND METHODS

Host–parasite lines

The snail and schistosome lines used here were descendents of those previously developed for Webster & Woolhouse's (1998) heritability study, wherein precise methodological details on host–parasite maintenance and the artificial selection protocol utilized can be obtained. In brief, 2 strains of the normally susceptible *B. glabrata* snails – one Vespiano (V) originally from Brazil and the other from Puerto Rico (PR), were artificially selected for either resistance (R) or susceptibility (S) to either of 2 *S. mansoni* parasite strains – one originally from Puerto Rico (pr) (from a different area from the snail population) and the other from Kenya (k). Unselected control lines were maintained in order to detect any change in compatibility status. Each replicate line was maintained in large (51×31 cm) tanks containing $\geqslant 60$ snails of matched compatibility selection status, with no intervention on mating strategy incorporated. By the F3 generation, infection prevalence was approximately 75% among susceptible-selected snail lines and 25% among resistant-selected snail lines following exposure to the same parasite strain to which their compatibility status had been selected. Infection prevalence remained at approximately 50% among unselected control snail lines, exposed to either parasite strain, across all generations. Likewise, due to the strain-specificity of compatibility, infection prevalence was approximately 50% amongst both resistant- and susceptible-selected snails exposed to a novel parasite strain (to which their compatibility status had not been selected toward), and hence was not significantly different to that of the unselected controls (see Webster & Woolhouse, 1998).

Experiment 1 : test for dominance

For clarity's sake, we refer to P1 and F1s here – however, the generations were in fact F4s and F5s from the artificially selected lines described above. In order to test whether resistance was dominant to susceptibility, following simple Mendelian inheritance, the following 7 steps were performed (see also Fig. 1).

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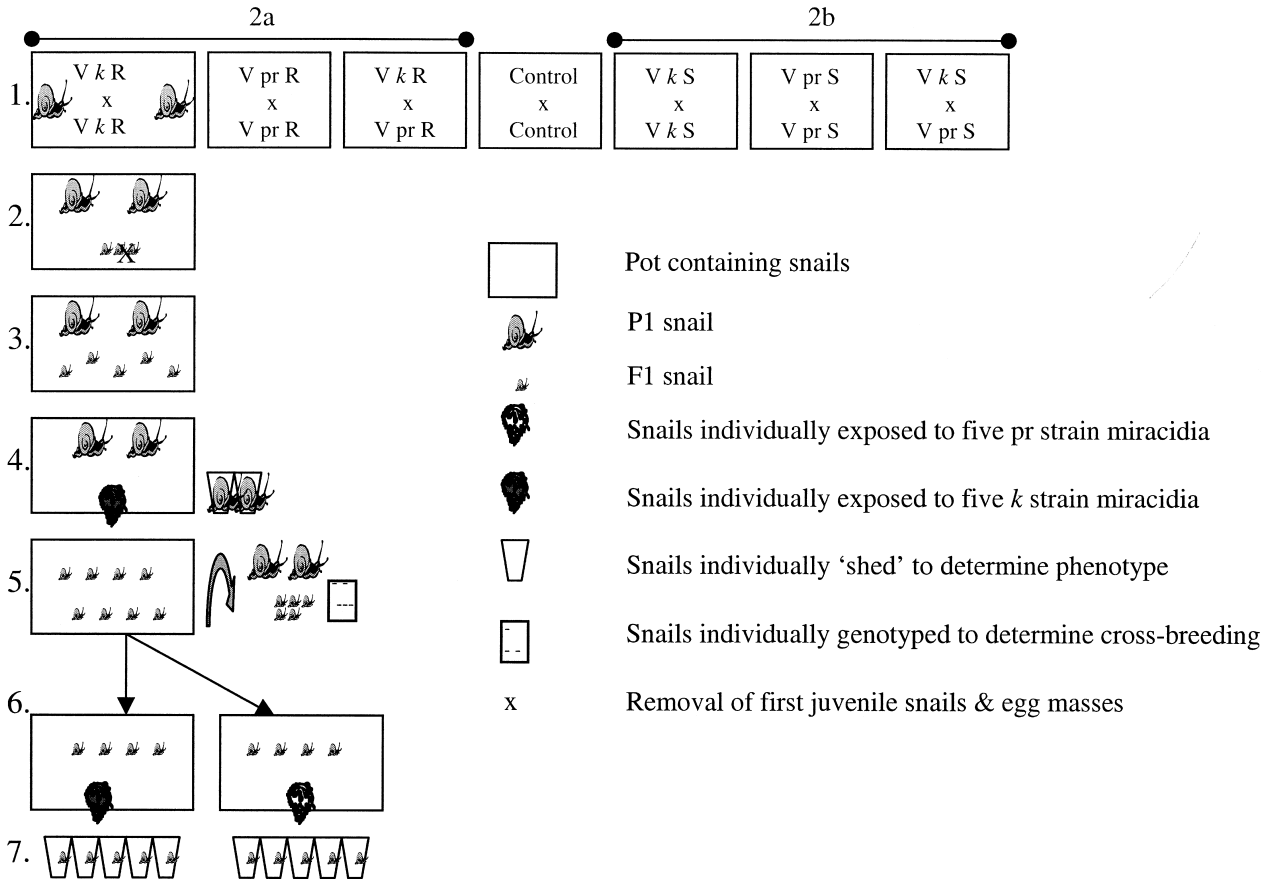

Fig. 2 Methodology used to test for Multi-loci of Compatibility (Exp. 2a and b). Details as for Fig. 1 except for inclusion of k for the Kenyan parasite strain.

(1) Pairs of adult P1 snails (post-onset of egg laying) were placed in small pots (14 × 8 cm), in the following combinations (using the abbreviations described above where e.g. PR pr R refers to a Puerto Rican snail strain (PR) artificially-selected to be resistant (R) towards a Puerto Rican parasite strain (pr). **Exp. 1a**: $n \geqslant 14$ pairs each of: PR pr R × PR pr R; PR pr S × PR pr S; & PR pr R × PR pr S. **Exp. 1b**: $n \geqslant 14$ pairs each of: V pr R × V pr R; V pr S × V pr S; & V pr R × V pr S.

Thus in the first two groups of both 1a and b, the compatibility status of each member of the pair was matched. In contrast, in the third groups one member of each pair was from a resistant-selected line (R) and the other from a susceptible-selected line (S). Finally, a group of $n \geqslant 14$ pairs of unselected V × V snails were also included to serve as an additional control against any potential inbreeding effects or selection bias within the artificial selection protocol.

By using paired snails here, in contrast to that used for the Webster & Woolhouse (1998) study, reproductive strategies were restricted to either outbreeding with the only available partner or selfing. $N \geqslant 14$ pairs (maximum starting sample size was $n = 20$ pairs per group) of snails in each were used in order to standardize final results to, wherever possible, the first 14 pairs surviving, cross-breeding, reproducing, and of the correct compatibility status – see below.

(2) All egg masses laid within initial 2 weeks following pairing were removed, in order to control against potential sperm storage (Paraense, 1956; Richards, 1970).

(3) Remaining egg masses were allowed to hatch and F1s to mature. Each pot contained Styrofoam sheets onto which snails preferentially lay their egg masses.

(4) The original P1 pairs were then individually exposed to 5 miracidia of the strain to which they had previously been selected (i.e. pr in Exp 1a and b). Five miracidia was chosen as this is the quantity required to result in a 50% infection status in these unselected *B. glabrata* lines (Webster & Woolhouse, 1998). Snails were then screened weekly 4–8 weeks later for cercarial shedding (by keeping the snails in darkness for 48 h and then exposing them, at 10.00 h, in vials containing 25 ml of dechlorinated water, for 2 h to a bright (100 watt) overhead light source) to confirm compatibility status. Data were excluded from any snail found to be of the incorrect compatibility status i.e. susceptible in a resistant-selected pair or vice versa.

(5) At week 9 the P1s and a random sample ($n =$ 4–5 from each family) of F1s were screened (in duplicate wherever possible) using the Randomly

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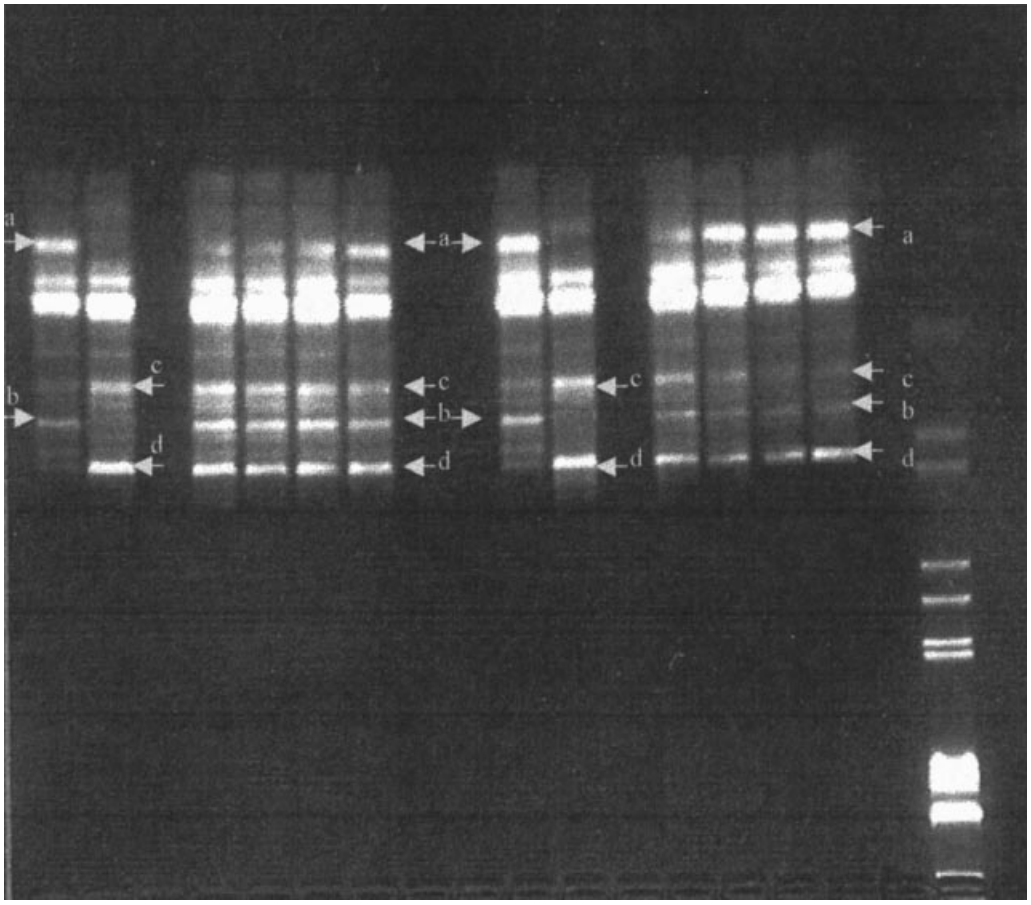

Fig. 3. Duplicate RAPD gels showing the distribution of polymorphic bands a–d in 2 parents (P1 and P2) and 4 offspring (01–04) from 1 family using primer 12. Bands a and b are present in P1 but absent in P2, whilst bands c and d are present in P2 but absent in P1. All bands a–d are present in all of the offspring indicative of cross-breeding. M represents the marker (Lambda DNA *ECO*RI *Hin*d III Digest (Sigma)).

Amplified Polymorphic DNA-PCR (RAPD) technique (Williams *et al*. 1990) to distinguish cross-bred from selfed offspring (Fig. 3). RAPDs provide the polymorphic genetic markers necessary for distinguishing self- from cross-fertilized individuals that lack other visible, Mendelian-inherited genetic markers. The DNA extraction and PCR amplification followed the protocol of Vernon, Jones & Noble (1995) which was designed to detect cross-breeding specifically amongst laboratory populations of *B. glabrata*. The only deviation from the procedure was that only primers 10 (TAGCAGCGGG), 12 (ATGGATCCGC) and 15 (CTGGCGGCTG) (R&D Technologies, Abingdon, UK) were used here. In brief, whenever a primer revealed one or more polymorphisms between the parents of a family, the DNA samples from the entire family sample (both P1s and $n = 4$–5 F1s) were amplified on the same PCR machine at the same time, and the amplification products run simultaneously on the same gel. Offspring that were the products of cross-fertilization would be expected to contain at least 1 band that was only found in 1 parent in addition to at least 1 band only found in the other parent (Fig. 3). In contrast, offspring from a self-fertilizing parent would show bands characteristic of 1 parent only. This is very important, as *B. glabrata* are facultative hermaphrodites (Vernon, 1993). Accordingly, as data on selfed offspring would be meaningless for this study (re objectives 1 and 2), families containing snails suspected to have selfed ($n = 6$) were excluded from analysis.

(6) In all suitable tanks (i.e. snails from the first 14 tanks (or as close to 14 as possible) in each group not excluded due to any of the factors described above), the remaining F1 offspring were exposed to 5 miracidia of the pr parasite strain (i.e. there were now 84 pots containing F1 snails from selected line crosses and a further 28 pots containing F1 snails from unselected line crosses).

(7) All F1 snails were shed once per week for 4–8 weeks post-exposure in order to determine compatibility phenotype status.

The results of Webster & Woolhouse's (1998) heritability and strain-specificity study on the same snail–parasite lines allow predictions to be made here (Table 1). As both Exps 1a and b represent replicates using different snail strains, we predict the same

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Table 1. Test for dominance of resistance: expected and observed percentage infected in PR and V snail strains

(PR = Puerto Rican snail strain; V = Vespiano snail strain; selected/exposed to its own familiar pr = Puerto Rican parasite strain; R = resistant-selected snail line; S = susceptible-selected snail line; C = unselected control snail line. Expected % +ve values are those predicted from Webster & Woolhouse (1998). As expected if resistance were a dominant trait, only the resistance phenotype (75 % infection rate) was observed in F1 crosses from matched resistant-selected P1 pairs and from non-matched pairs where one P1 was from a resistant-selected line and the other from a susceptible-selected. The susceptibility phenotype (25 % infection rate) was only observed in F1 crosses from matched susceptible-selected P1 pairs. (See text for further details.))

P1 cross ($n \leqslant 14$ pairs)	Parasite strain F1s exposed to		Expected % +ve	Observed % +ve
PR pr R × PR pr R	pr	own	25	29
PR pr R × PR pr S	pr	own	25	36
PR pr S × PR pr S	pr	own	75	81
V pr R × V pr R	pr	own	25	31
V pr R × V pr S	pr	own	25	36
V pr S × V pr S	pr	own	75	60
V C × V C	pr		50	54

Table 2. Test for multi-loci: expected and observed percentage infected in resistant-selected, susceptible-selected, and unselected control *Biomphalaria glabrata* snail lines exposed to either of 2 strains of *Schistosoma mansoni*

(Key as for Table 1, except where: own = the familiar parasite strain to which snail lines have been selected towards ('own' = where each member of the pair is selected towards different familiar parasite strains); novel = an unfamiliar parasite strain to which snail lines have not been selected towards; pr = Puerto Rican parasite strain; k = Kenyan parasite strain. (* Significantly raised mortality rate during the pre-patent period – see text for further details.) As expected if compatibility is a multi-loci trait, the resistance phenotype (75 % infection rate) was simultaneously observed against two parasite strains in F1 crosses arising from non-matched P1 pairs (where one was resistant-selected towards one parasite strain and the other resistant-selected to a second strain). The converse was also suggested amongst some susceptible-selected crosses (25 % infection rate). Strain-specificity of compatibility was observed, as all effects were lost if snails were exposed to a novel parasite strain (50 % infection rate, as for unselected controls).)

P1 cross ($n = 14$ pairs)	Parasite strain F1s exposed to		Expected % +ve	Observed % +ve
V k R × V k R	k	own	25	33
	pr	novel	50	56
V k R × V pr R	k	'own'	25	35
	pr	'own'	25	23
V pr R × V pr R	k	novel	50	46
	pr	own	25	31
V k S × V k S	k	own	75	78
	pr	novel	50	46
V k S × V pr S	k	'own'	75	43*
	pr	'own'	75	92
V pr S × V pr S	k	novel	50	52
	pr	own	75	60
V C × V C	k		50	47
V C × V C	pr		50	54

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trends across the two combinations. We would predict an approximate prevalence of 25% for the F1 resistant-selected homologous crosses exposed to the Puerto Rican (pr) parasite strain in 1a and b. Likewise, we would predict an approximate prevalence of 75% for the susceptible-selected homologous crosses. However, if resistance is dominant over susceptibility, and follows simple Mendelian inheritance, we would predict a 25% infection prevalence amongst the heterologous crosses, as in the F1 generation only the resistance phenotype would be displayed. Infection rates would be predicted to remain at approximately 50% amongst unselected control snails exposed to the pr parasite (Table 1).

Experiment 2a, b : test for multi-loci of compatibility

This experiment was designed (Fig. 2) to test whether snails could be cross-bred to be resistant and/or susceptible to more than 1 strain of *S. mansoni* (i.e. as contrasted to the single strain specific compatibility of the original artificially-selected lines).

(1) Pairs of adult P1 snails (post-onset of egg laying) from artificially selected snail lines were placed in small pots (14 × 8 cm) in the following combinations. **Exp. 2a**: $n \geqslant 14$ pairs each of: V k R × V k R; V pr R × V pr R; and V k R × V pr R. **Exp. 2b**: $n \geqslant 14$ pairs each of: V k S × V k S; V pr S × V pr S; and V k S × V pr S. Thus the first 2 groups consisted of matched snails in each pair. In contrast, in the third groups one member of each pair was of a compatibility status selected towards the Kenyan (k) parasite and the other was selected towards the Puerto Rican (pr) parasite. Finally as in the previous experiments, the group of $n \geqslant 14$ pairs of unselected V × V snails were also included to serve as controls.

The same (steps 2–5) protocol as for Exps 1a and b above were then followed. However, here (step 6), in all suitable tanks (i.e. snails from the first max.14 tanks in each group not excluded due to any of the factors described above), the remaining F1 offspring were then equally divided into 2 fresh (14 × 8 cm) pots. All the F1 snails in one pot were individually exposed to 5 miracidia of the k parasite strain and all the F1 snails in the other pot were exposed to 5 miracidia of the pr parasite strain (i.e. for Exps 2a and b there were now a maximum of 168 pots containing F1 snails from selected lines and a further 28 pots containing F1 snails from unselected lines). Finally (point 7), all F1 snails were shed once per week for 4–8 weeks post-exposure in order to determine compatibility phenotype status.

As above, the results of Webster & Woolhouse's (1998) heritability and strain-specificity study allowed us to predict the results here (Table 2). For Exp. 2a one would predict an approximate prevalence of 25% for the first 2 groups when exposed to their familiar parasite strain, and 50% when exposed to the novel parasite strain. However, if snails were cross-bred to be resistant to both parasite strains (and hence $\geqslant 2$ independent loci are involved), we would predict here an approximate 25% prevalence when exposed to either parasite strain. In contrast, infection rates would remain at approximately 50% to either parasite strain amongst unselected control snails.

For the susceptible-selected snails of Exp. 2b, the same patterns are predicted, except with a 75% infection rate amongst snails exposed to their familiar parasite strain (and 50% to the novel strain) in the former 2 groups. If snails were cross-bred to be susceptible to both strains, we would predict a 75% infection rate to both parasites in the third group (Table 2).

Statistical analyses

χ^2 tests were performed and used to compare observed infection prevalence results here from that predicted from the artificial-selection (a-s) procedure of Webster & Woolhouse (1998). Contingency tables compared observed results between experimental groups here. In order to test for any differences in mortality between the groups or crosses during the pre-patent period (i.e. before each phenotype could be assessed), individual χ^2 analyses were used on the number of snails surviving/not surviving per experimental group and compared to the number of snails surviving/not surviving per unselected control group, corrected for multiple tests using Boneferroni's correction (making a cut-off of $P \leqslant 0{\cdot}002$).

Snail genotype is referred to throughout as either homologous or heterologous relating to the cross used from artificially selected snail lines. The snail phenotype refers to the observed compatibility status (i.e. whether a snail was found to be shedding cercariae at any point 4–8 weeks post-exposure to 5 miracidia or not).

Percentage prevalences reported (e.g. 75% resistance) should not be interpreted as meaning that each snail is 75% resistant, but rather that 25% of the snails exposed in that line/group were infected and 75% remained uninfected post-exposure to 5 miracidia, reflecting gene frequencies in the population and a variety of potential gene combinations.

RESULTS

There was no significant difference in mortality rate during the pre-patent period between any of the experimental groups from that of the unselected control groups in Exps 1a, b or 2a. However, 1 heterologous cross-group within Exp. 2b did show

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a significantly higher mortality level than that of the controls ($\chi^2 = 18{\cdot}69$, D.F. $= 1$, $P = 0{\cdot}0001$: see Table 2).

Exp. 1a and b : test for dominance

There were no significant differences between families within any group (for the $n \leqslant 14$ families per group in Exps 1a and b), and thus the data were pooled. Infection rate remained at approximately 50% amongst unselected V snail (47%) snails exposed to the pr parasite.

Table 1 shows that the observed percentage infection prevalence was approximately matched to that predicted from the artificially selected lines if resistance were dominant over susceptibility overall ($\chi^2 = 5{\cdot}80$, D.F. $= 5$, $P = 0{\cdot}32$) and for each snail strain separately (PR $\chi^2 = 0{\cdot}9$, D.F. $= 2$, $P = 0{\cdot}64$; V $\chi^2 = 4{\cdot}27$, D.F. $= 2$, $P = 0{\cdot}12$).

Thus, there were no significant differences in the resistance phenotype observed (infection prevalence) between the homologous resistant-selected crosses and the resistant-selected/susceptible-selected heterologous crosses overall ($\chi^2 = 0{\cdot}78$, D.F. $= 1$, $P = 0{\cdot}37$), or for each snail strain separately (PR $\chi^2 = 0{\cdot}50$, D.F. $= 1$, $P = 0{\cdot}47$; V $\chi^2 = 0{\cdot}29$, D.F. $= 1$, $P = 0{\cdot}37$). In contrast, the infection prevalence was higher amongst the homologous susceptible-selected crosses from that of the heterologous crosses overall ($\chi^2 = 13{\cdot}63$, D.F. $= 1$, $P = 0{\cdot}0002$), and for each snail strain separately (although this just failed to reach significance amongst the V snails: PR $\chi^2 = 11{\cdot}10$, D.F. $= 1$, $P = 0{\cdot}009$; V $\chi^2 = 3{\cdot}36$, $P = 0{\cdot}06$).

The results thus indicate that resistance is dominant over susceptibility.

Exp. 2a and b : test for multi-loci

Table 2 shows that the observed percentage infection prevalence amongst resistant-selected crosses was approximately matched to that predicted from the artificially selected lines ($\chi^2 = 2{\cdot}75$, D.F. $= 5$, $P = 0{\cdot}73$).

As for Webster & Woolhouse's (1998) study, the strain-specificity of resistance was demonstrated in that the infection prevalence amongst homologous familiar parasite crosses was significantly lower than amongst homologous novel parasite crosses ($\chi^2 = 13{\cdot}63$, D.F. $= 1$, $P = 0{\cdot}0002$), the latter being not significantly different from that of the unselected control snails exposed to the same parasite strains ($\chi^2 = 0{\cdot}21$, D.F. $= 1$, $P = 0{\cdot}64$).

However, in this study, support of multi-loci involvement was also suggested as the heterologous crosses (artificially selected snails cross-bred to be resistant to both parasite strains) showed no significant differences in infection prevalence phenotype rate from that of the homologous familiar parasite crosses ($\chi^2 = 0{\cdot}02$, D.F. $= 1$, $P = 0{\cdot}89$), but were significantly lower than that from the homologous novel parasite crosses ($\chi^2 = 10{\cdot}20$, D.F. $= 1$, $P = 0{\cdot}001$).

Unfortunately, due to significantly elevated mortality levels within the k exposed heterologous cross-group ($\chi^2 = 18{\cdot}69$, D.F. $= 1$, $P = 0{\cdot}0001$: see Table 2) the results obtained in Exp. 2b were not as robust as those obtained in Exp. 2a. Nevertheless, as the same overall trends occurred, we feel justified to present our results here. Table 2 shows that the observed percentage infection prevalence amongst susceptible-selected crosses was approximately matched to that predicted from the artificially selected lines ($\chi^2 = 9{\cdot}29$, D.F. $= 5$, $P = 0{\cdot}10$).

Strain-specificity of susceptibility was suggested in that the infection prevalence amongst homologous familiar parasite crosses was significantly higher than amongst homologous novel parasite crosses ($\chi^2 = 4{\cdot}51$, D.F. $= 1$, $P = 0{\cdot}03$), the latter being not significantly different from that of the unselected control snails exposed to the same parasites ($\chi^2 = 0{\cdot}02$, D.F. $= 1$, $P = 0{\cdot}88$).

Furthermore, support of multi-loci involvement was demonstrated as the heterologous crosses (artificially selected snails cross-bred to be susceptible to both parasite strains) showed no significant differences in infection prevalence phenotype rate from that of the homologous familiar parasite crosses ($\chi^2 = 0{\cdot}06$, D.F. $= 1$, $P = 0{\cdot}80$), but was significantly higher than that from the homologous novel parasite crosses ($\chi^2 = 5{\cdot}13$, D.F. $= 1$, $P = 0{\cdot}02$).

Reproductive strategy

Six families (5%), out of a total of 122 families tested contained snails that were suspected to have been the result of self-fertilization in Exp. 1a and b, and 8 (7%) out of a total of 110 families tested in Exp. 2a and b. All selfed individuals were from resistant-selected homologous crosses.

DISCUSSION

The overall results presented here provide support to the hypotheses that *B. glabrata–S. mansoni* compatibility, at least for the 2 snail–parasite strain combinations used here, is under the genetic influence of at least a 2 loci 2 allele model, with resistance dominant over susceptibility, and where genotype may influence sexual strategy.

For both snail strains within Exp. 1a and b, there were no significant differences between the homologous and heterologous resistant crosses in their compatibility phenotypes, indicative of a dominant trait (Richards, 1975*a*, *b*; Fritz & Simms, 1992; Behnke *et al*. 2000). Within Exp. 2a, homologous resistant-selected snail crosses exposed to their

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familiar parasite strain (to which their compatibility status had been selected), showed significantly lower infection rates than those snails exposed to the novel parasite strain, indicative of the same strain specificity of compatibility as previously reported by Webster & Woolhouse (1998). However, here, heterologous crosses from parents selected to be resistant to different parasite strains showed approximately matched infection rate phenotypes with that of the homologous resistant-selected crosses. Within Exp. 2b, significantly increased mortality in some snails, as is common amongst *S. mansoni*-exposed *B. glabrata* (Webster & Woolhouse, 1999), weakened the effect. Nevertheless, both strain specificity and evidence of independent multi-loci involvement, at least amongst snails exposed to the Puerto Rican parasite strain, were also indicated. This thus demonstrates, to the author's knowledge for the first time, that snails may be experimentally bred to share compatibility with more than 1 parasite strain. In doing so, these data may thereby provide further empirical support for the matching allele at multiple loci models of co-evolution in this system (Frank, 1996).

Such potential for compatibility polymorphism, even between single populations and generations through cross-breeding, may have both epidemiological implications for schistosome transmission (Woolhouse, 1996; Hoffman *et al*. 1998; Davies *et al*. 1999), and again for co-evolutionary theory. Most applicable may be that of the geographical mosaic theory of co-evolution, where such rapid evolutionary changes within species can lead to geographical variation in the nature of a host–parasite interaction (Thompson, 1994), and/or that of the Red Queen hypothesis, which relies on time-lagged frequency-dependent selection by parasites against host genotypes (Hamilton, 1980; Hamilton *et al*. 1990). Indeed, the Red Queen hypothesis also predicts that when there is a high potential risk of virulent parasitic infection, as would be the case amongst the susceptible-selected snails here, cross-breeding would be favoured over selfing. This is explicable as genotypic diversification amongst sexually reproduced progeny may help them evade co-evolving parasites (Hamilton, 1980; Hamilton *et al*. 1990; Bell, 1982; Jaenike, 1978). Likewise, cross-breeding may be further selected for in mixed-mating systems such as this where inbreeding depression is marked (Howard & Lively, 1994), as has also been reported in this system (Vernon, 1993; Vernon *et al*. 1995). Molecular markers were used here to determine the reproductive strategy within single strains of *B. glabrata* (previous studies, such as those by Richards (1970, 1975*a*, *b*), relied instead on phenotypic markers from crosses between pigmented and non-pigmented snail strains). In accordance with the aforementioned predictions, the majority (94%) of offspring were the consequence of cross- rather than self-fertilization. This is also consistent with earlier studies on unselected *B. glabrata* snail lines (Paraense, 1956; Vernon *et al*. 1995 – the latter of which found 100% cross-fertilization rates as revealed by RAPD markers). However, it may be of interest to note that, although frequencies may be too low for any firm conclusions to be drawn, of those progeny identified to be the consequence of selfing here, all were from resistant-selected homologous crosses, and hence only those snail lines unlikely to become infected by the parasite. Similar findings have been found in field populations of other snail–trematode systems, where selfing occurs, and can even replace cross-breeding, but only when the likelihood of parasitic infection is rare (Lively, 1987). Thus one could speculate that, not only is the frequency of sexual individuals positively correlated with the frequency of individuals actually infected by (rather than simply exposed to) the parasite, but also that resistant-selected snails may prefer to self in order to prevent out-breeding depression, where there is the potential break up by recombination of successful co-adapted gene complexes (Bateson, 1983). Such a proposition need not be surprising considering the strong empirical support for active mate choice within various *Biomphalaria* spp. on the basis of habitat, nutritional or parasite status (Vernon, 1993; Rupp, 1996; Rupp & Woolhouse, 1999).

Nevertheless, the situation remains complex. The Red Queen focuses on cross-breeding for the production of rare offspring to increase their chance of escaping infection from the local co-evolving parasite (Lively, 1987). Hence resistance may simply be characterized as a temporary phenomenon conferred by having been rare in the recent past. However, Exp. 2a suggests the importance of sexual reproduction in producing progeny actively resistant (i.e. not simply rare) to more than 1 strain of *S. mansoni*. Moreover, (though the results were less robust) the converse also appeared to be true, in that cross-breeding could result in progeny being susceptible to more than 1 parasite strain. Thus for the latter group, as schistosomes are highly virulent parasites, cross-breeding can, under certain circumstance, incur subsequent costs (Lloyd & Lively, 1993) – in this case by producing progeny with a high probability of subsequent infection from either parasite strain. One explanation may lie with another major assumption of co-evolutionary theory, that being of the potential cost of resistance (Frank, 1994; Fritz & Simms, 1992). Such a cost, in terms of reduced fertility, has recently been reported in this system (Webster & Woolhouse, 1999). Thus the results here may add further support to the idea that there may also be strong selection for susceptible snails under some circumstances, namely in the absence of parasite pressure. Indeed, it may be interesting for future studies to determine whether

there is any additive effect on fertility amongst strains of snails artificially selected to be either resistant or susceptible to more than 1 strain of parasite.

To conclude, these data suggest for this *B. glabrata–S. mansoni* combination, that resistance is a dominant, strain-specific, and multi-loci trait, which may also have implications for mixed mating strategies. Such results may help pave the way for future molecular studies aimed to elucidate the genetic basis of snail–schistosome compatibility. These should not be restricted to *B. glabrata–S. mansoni* systems alone, as other snail–trematode systems (Berrie, 1970; Wright, 1973; Lively & Dybdahl, 2000), as well as numerous unrelated host–parasite systems (Wakelin & Blackwell, 1988), all show similar compatibility polymorphisms, and all of which may provide ideal models on which to empirically test the assumptions of current co-evolutionary theory.

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