Coevolution and compatibility in the snail–schistosome system

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SUMMARY

In stark contrast to the huge body of theoretical work on the importance of hosts and parasites as selective agents acting on each other, until recently, little systematic empirical investigation of this issue has been attempted. Research on snail–schistosome interactions have, therefore, the potential for making an important contribution to the study of coevolution or reciprocal adaptation. This may be particularly pertinent since snail–schistosomes represent an indirectly transmitted macroparasite system, so often overlooked amongst both theoretical and empirical studies. Here we review ideas and experiments on snail–schistosome interactions, with particular emphasis on those that may have relevance to the potential coevolution between host resistance and parasite infectivity and virulence. We commence with an introduction and definition of the general concepts, before going into detail of some specific studies to illustrate these: evidence of snail–schistosome coevolutionary process in the field; evidence of coevolutionary processes in the laboratory; a general assessment of the applicability of coevolutionary models in snail–schistosome interactions; and finishing with a section on conclusions and areas for further study.

Key words: Coevolution, compatibility, resistance, virulence, infectivity, snail, schistosome.

INTRODUCTION

There is a huge body of theoretical work on the importance of hosts and parasites as selective agents acting on each other. However, there has until recently been little systematic empirical investigation of this topic. One area in which research on snail–schistosome interactions has the potential for making an important contribution is, therefore, the study of coevolution or reciprocal adaptation. In particular, the evolution of snail resistance and schistosome infectivity and virulence may offer the prospect of an insight into the genetics of adaptation.

One approach from which to infer snail–schistosome coevolution is to investigate the current 'end points' of coevolutionary interactions in the field. An alternative approach is to demonstrate coevolution in action through controlled laboratory experiments. The ultimate demonstration may be longitudinal field studies that incorporate both these factors. There are several ways to achieve these aims. The first way is to document additive genetic variation in host resistance, parasite infectivity and/or virulence using quantitative genetic techniques or artificial selection experiments. This may help elucidate the genetic architecture underlying these traits. One must also show that host resistance affects parasite fitness and conversely that parasite infection and virulence affect host fitness (Kraaijeveld *et al*. 1998). If genetic variability is demonstrated, one must then identify how such variability is maintained in natural populations, and whether it varies at different times or places. Likewise, one should also consider whether evolutionary changes in resistance, infectivity or virulence influence host-parasite population dynamics.

Our aims here are to review ideas and experiments on snail–schistosome interactions, with particular emphasis on those that may have relevance to the potential coevolution between host resistance and parasite infectivity and/or virulence. Much of this paper concerns examples from *Biomphalaria glabrata–Schistosoma mansoni* interactions, as this is the system that has been most intensively investigated. Nevertheless, an attempt is made to put this work within a broader snail–trematode framework.

The review will be split into five sections. We commence with an introduction and definition of the general concepts, before going into detail of some specific studies to illustrate these: evidence of snail–schistosome coevolutionary process in the field; evidence of coevolutionary processes in the laboratory; a general assessment of the applicability of coevolutionary models in snail–schistosome interactions; and finally finishing with a section on conclusions and areas for further study. Several other issues, such as the physiological basis of resistance and/or infectivity, are of undoubted relevance to understanding this system, but are outside the scope of our account. Moreover, these aspects will addressed in detail in other parts of this issue (see e.g. de Jong-Brink; Lewis, Patterson & Richards, both this supplement).

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COEVOLUTION: DEFINITIONS AND MODELS

Before progressing further, it is appropriate to define the terms we will use in this account, given that there are inconsistencies across the literature.

Co-evolution

Co-evolution is evolution in one species in response to selection imposed by a second species, accompanied by evolution in the second species in response to reciprocal selection imposed by the first species. Host–parasite coevolution is driven by the reciprocal evolution of host resistance and parasite infectivity and/or virulence (Sorci, Moller & Boulinier 1997). However, reciprocal selection has seldom been measured in natural, particularly animal, populations (Clayton *et al*. 1999).

Resistance

The term 'resistance' (sometimes referred to in the literature as 'non-susceptibility' or 'refractoriness') specified here refers to the genetic, biochemical and/or physiological profiles that inhibit parasite establishment, survival and/or development within the host (Coustau, Chevillon & Ffrench-Constant, 2000).

Within this definition two forms of host resistance may be considered (Gandon & Michalakis, 2000). First, the host could adopt a quantitative form of resistance, which can be used to limit the deleterious effects induced by the parasites. In this case, hosts may be infected by the parasites but more resistant ones are harmed less. Alternatively, the host could adopt a qualitative form of resistance that would prevent any infection by the parasite (i.e. resistant hosts cannot be infected at all). The majority of models of host–parasite coevolution regard 'resistance' to be the qualitative type referred to here, and will be the major focus of this article.

Infectivity

Infectivity is defined as the infective capacity of the pathogen when applied to suitable host tissues. Infectivity can variably be thought of as the number or type of host genotypes a parasite is capable of infecting (Read, 1994) or, at the population level, the proportion of a particular host strain the parasite will infect. In snail–schistosome interactions, an infected host is usually considered to be one that produces the next infective stage, the cercariae (Wright, 1974).

Virulence

Virulence may be defined as the reduction in host fitness (lifetime reproductive success) attributable to parasitic infection (Read, 1994). Parasite-induced host mortality, a commonly used measure of virulence in evolutionary models, may be a direct or indirect result of parasitic infection. Examples of the latter include increased predation risk, susceptibility to other pathogens, or decreased competitive ability (Poulin, Hecker & Thomas, 1998). Virulence, by definition, also represents a fitness cost to most parasites, since parasites are intimately dependent on their hosts for reproduction and survival (Combes, 1997). For example, killing the host presents an obvious evolutionary cost to the parasite, at least for those parasites where host death prevents further parasite propagation and transmission.

Definitions of virulence, however, do differ across the literature. Although we will use that presented above, it is worth considering a commonly used alternative: the infective capacity of pathogens (here termed 'infectivity'). This is the usage of virulence in many models of host–pathogen coevolution that have been developed primarily for plant–pathogen systems (e.g. Frank, 1993). It is interesting to note that such models implicitly assume a positive association between 'infectivity' and 'virulence' (the severity of the infection). However, this is not always the case (e.g. Barbosa, 1975; Ebert, 1994). Moreover, these factors are not equivalent *vis à vis* cost-benefit evolution (see below). It is therefore important to distinguish between the two in this review.

Compatibility

Virulence, infectivity and resistance are complex features of host–parasite interactions. Though they may appear to be attributes of the parasite or of the host, they are in fact the net effect of the physiological, morphological and behavioural interactions between parasite and host (Toft & Karter, 1990). Thus it should always be noted that definition of a host as resistant or a parasite as having high infectivity or virulence, may be specific to a particular host–parasite species or strain combination. The relationship may thus be better described as 'compatibility'.

As regards snail–schistosome interactions, in compatible interactions, the parasite recognizes, penetrates and develops within the snail, giving rise to the parasites next infective stage, the cercariae. Alternatively, in incompatible interactions, the larval trematode either fails to recognize, penetrate or develop in the snail, or penetrates and is recognized as non-self, and is destroyed by the mollusc's internal defence system (van der Knapp & Loker, 1990).

Coevolutionary models

Coevolution may take different forms and several models have been proposed. Some of the most important ones, described below, may have relevance to snail–schistosome co-evolution, but even these are not mutually exclusive for a coevolving system.

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Frequency-dependent or 'Red Queen' coevolution. To understand the kind of selection pressure acting on resistance, infectivity and virulence, it is important to understand the specificity of the interaction. This question is important, as specificity will give rise to adaptation to the most common genotype and hence frequency-dependent advantage for rare genotypes. The result is locally dynamic or 'Red Queen' coevolution with a constant flux of genotype frequencies and high heritability of infectivity, virulence and resistance (Kraaijeveld & Godfray, 1999; see also Lively, this supplement). In models of dynamic coevolution of host resistance and parasite infectivity (Morand, Manning & Woolhouse, 1996), parasite genotypes track common host genotypes, promoting a frequency-dependent advantage to rare host genotypes, since they escape the deleterious consequences of parasitic infection. The 'rare' advantage of a host genotype will depend not only on its likelihood of being infected (parasite infectivity), but also on the severity of any such infection (virulence) (Lively, 1999). Just as genotype-specific resistance, infectivity and virulence mechanisms lead to a constant turnover of gene frequencies, so species-specific resistance, infectivity and virulence may lead to changes in species frequency (Kraaijeveld & Godfray, 1999).

Density-dependent coevolution. Non-specific resistance and infectivity involves increased resistance improving survival against all genotypes of a parasite, and increased infectivity improving performance against all genotypes of host, such that the rank order of resistance of different host strains exposed to different parasite strains (or of infectivity to different hosts) will be constant. Frequency-dependent dynamics and Red Queen coevolution are thus not expected. However, where parasites have a major effect on host population dynamics, coupled coevolution could still lead to spatio-temporal variation in resistance, infectivity and virulence. In this situation, higher parasite densities may lead to selection for enhanced resistance, which might then cause either a reduction in parasite numbers (and hence a relaxation of selection for resistance) or a corresponding increase in the level of infectivity. Virulence is again important as it determines the strength of selection on hosts (Lively, 1999). Models of this type of interaction in host–parasitoid interactions that assume density-dependent rather than frequency-dependent selection suggest that the population and genetic dynamics may reach an equilibrium or show persistent cycles, depending on the initial conditions (Kraaijeveld & Godfray, 1999).

Cost-benefit trade-offs and locally fixed optima. Where frequency-dependent or density-dependent selection does not occur, host-coevolution might lead to locally fixed optima for the levels of resistance, infectivity and virulence, which may vary across time or space as costs and benefits change. Genetic constraints are considered to be fundamental in life-history evolution (Messenger, Molineux & Bull, 1999). While increased resistance or infectivity is clearly beneficial in the context of host–parasite interactions, there may be trade-offs involving other components of fitness that might cause the optimum level of either trait to be lower than the maximum achievable. Thus, for example, host resistance may bear metabolic costs that are higher than the increases in fitness achieved through avoiding parasite infection (Frank, 1994). This might be particularly true where parasite prevalence is low. Virulence represents a fitness cost to most parasites, since parasites are intimately dependent on their hosts for their reproduction and survival (Combes, 1997). Thus the fitness cost of being virulent may influence the direction of parasite evolution (see below).

The geographic mosaic theory of coevolution. The geographic mosaic theory of coevolution differs slightly from that of the models described above in that it is a general hypothesis about how the raw materials of coevolution are organized (Thompson, 1994, 1999). It suggests that there is a selection mosaic among populations, favouring different evolutionary directions to interactions in different populations. Thus for example, variations in opportunities for transmission or parasite-independent mortality (Ebert & Herre, 1996), and environmental effects on the expression of host resistance (Abdullah, 1997) are expected to alter the balance of cost-benefit trade-offs governing the evolution of parasite virulence (Kraaijeveld & Godfray, 1999). The geographic mosaic theory further assumes that there are 'coevolutionary hotspots', such that reciprocal selection need not occur in all populations. Finally, the hypothesis suggests that there is a continuous remixing of the range of coevolving traits, resulting from the mosaic, gene flow, random genetic drift and the local extinction of populations (Thompson, 1994, 1999).

EVIDENCE OF SNAIL–SCHISTOSOME COEVOLUTION IN THE FIELD

Variability in host–parasite compatibility may be taken as evidence of coadaptation and, in some cases, potential coevolution in natural populations. Snail–trematode compatibility is a highly specific relationship, often at the population or strain levels for both participants (Lo & Lee, 1995; Webster & Woolhouse, 1998). This specificity has the important practical effect of limiting medically important trematodes such as schistosomes to geographic areas occupied by compatible snails (van der Knaap & Loker, 1990).

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Whilst variations in snail–schistosome compatibility was first reported by Files & Cram (1949), perhaps the clearest example of the presence of compatibility factors in both snail and schistosome is provided by Paraense & Correa (1963), who showed that a *S. mansoni* strain adapted to *Bi. tenagophila* will not infect *Bi. glabrata*, and vice versa. Within species, Manning, Woolhouse & Ndamba (1995) used a reciprocal cross-infection design with *Bulinus globosus* snails and *Schistosoma haematobium/mattheei* from Zimbabwe and found that sympatric parasite–host combinations were more compatible than allopatric combinations across two sites 60 km apart. Similar findings have also been suggested by Lively (1989) and Lively & Dybdahl (2000) for *Microphallus* spp. infections of *Potamopygrus antipodarum* in New Zealand. However, not all studies demonstrate such local adaptation (see Morand *et al*. 1996). For example, Vera *et al*. (1990) also used reciprocal cross infection experiments of wild *Bu. truncatus* snails and *S. haematobium* parasites, and did not find any difference in compatibility across three sites up to 800 km apart. Several other studies which tested the infectivity of a single trematode population to two or more snail populations also found exceptions to this rule (reviewed by Richards & Shade, 1987; Morand *et al*. 1996). Morand *et al*. (1996) therefore used field data taken from the literature to develop a mathematical model based on the dynamics of the host–parasite interaction. In the model, parasite infectivity and host susceptibility were defined by the matching of genotypes in a diploid system, and it was shown that frequency-dependent coevolution could explain such local adaptation. They further demonstrated that whilst there is a tendency for sympatric combinations to be more compatible than allopatric combinations, instances of the reverse pattern also occur. This may be explained by the fact that frequency-dependent models of host–parasite coevolution, such as that of the Red Queen hypothesis, predict that changes in sympatric parasite allele frequencies tend to lag behind host allele frequencies (Lively & Apanius, 1995; Morand *et al*. 1996). Thus allopatric parasite allele frequencies may therefore, by virtue of being in a different phase of the cycle, chance to correspond more closely to host allele frequencies (Woolhouse & Webster, 2000).

EVIDENCE OF SNAIL–SCHISTOSOME COEVOLUTION IN THE LABORATORY

The results of the compatibility studies described above may suggest snail–schistosome coevolution. Further substantiation, nevertheless, requires complimentary investigation in the laboratory, where phenotypic and other variables can be tightly controlled.

Heritability of host resistance

Models of host–parasite coevolution require that variation in host resistance to parasite infection and of parasite infectivity and/or virulence is, at least partially, genetically determined (Anderson & May, 1982), since heritable genetic variation is a prerequisite for natural selection. Richards and colleagues have made extensive studies of the genetics of resistance and susceptibility of *Bi. glabrata* to *S. mansoni*. As these are reviewed in a separate chapter of this issue (Lewis *et al*. this supplement), we will not duplicate information here. Instead we will focus on other studies aimed to elucidate the heritability of host resistance.

Webster & Woolhouse (1998) used artificial selection experiments to determine the heritability of snail–schistosome compatibility. Two unselected populations of *Bi. glabrata* snails, and two unselected *S. mansoni* parasite populations, were chosen for artificial selection (see Fig. 1). For each snail–schistosome strain combination, adult P_1 snails were individually exposed to five *S. mansoni* miracidia and subsequently divided up into groups containing either uninfected 'resistant-selected' or infected 'susceptible-selected' individuals. F_1 progeny were then exposed to the same strain of *S. mansoni* as their parents and only snails consistent with their selection group were maintained. This breeding and selection protocol was continued until the F_3 generation. A matched number of unselected control snails were also exposed to both parasite strains at each generation.

The results suggested that compatibility in this system is heritable. Snails, of either strain, selected for resistance were significantly more resistant than controls to *S. mansoni* by the F_1 generation. Likewise, snails selected for susceptibility were significantly more susceptible than controls to *S. mansoni*, although increased susceptibility did not develop before two generations of selection. By the F_3 generation, infection prevalence was approximately 25% among resistant-selected snail lines and 75% among susceptible-selected snail lines. Unselected control snail lines remained at approximately 50% infection rate when exposed to five miracidia per snail throughout each generation (Fig. 2).

A subsequent study on these same host–parasite lines (Webster, 2001) then investigated whether such resistance is dominant over susceptibility, following simple Mendelian inheritance, as is common for many plant–pathogen (Fritz & Simms, 1992) and other animal–helminth (Richards, 1975*a*,*b*; Behnke *et al*. 2000) interactions. Individual adult snails from each artificially-selected replicate snail line described above were paired in small pots with a selected partner. In the first two groups (of $n \geqslant 14$ pairs each) the compatibility status of each member of the pair

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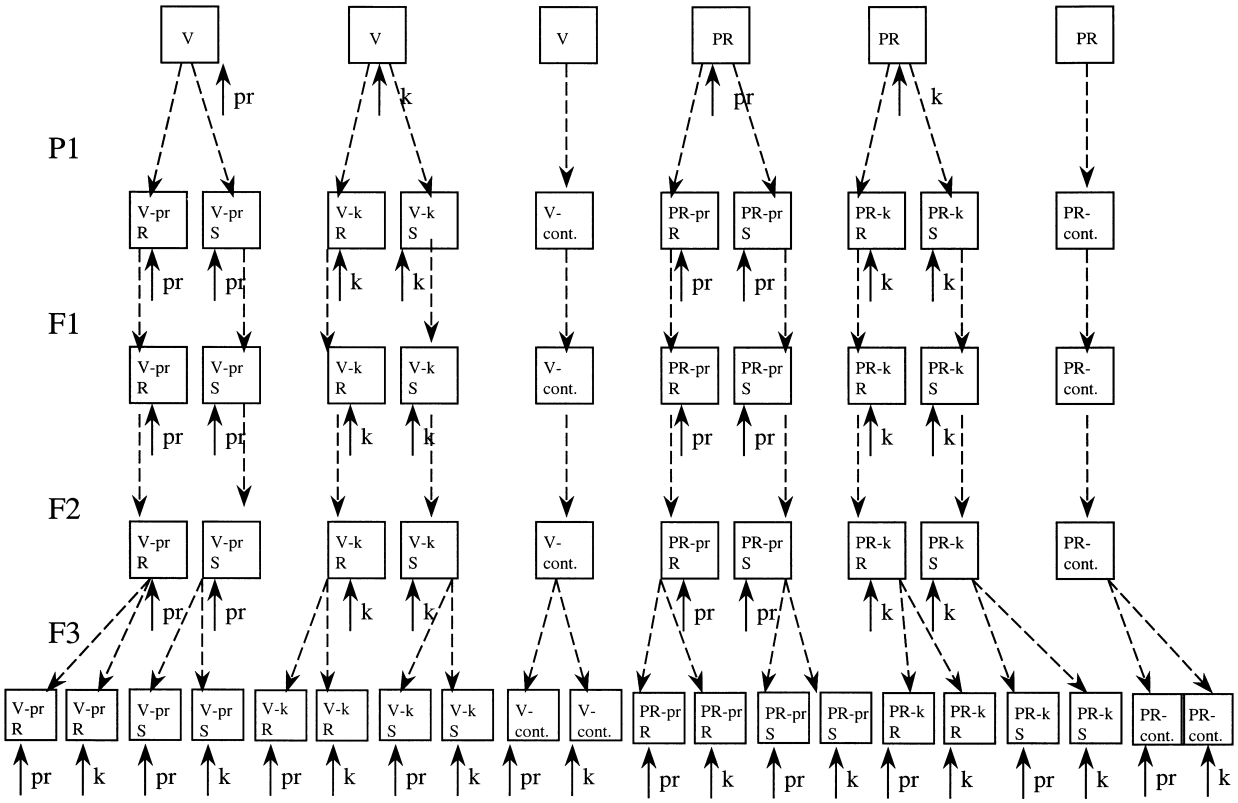

Fig. 1. Artificial selection methods for *Biomphalaria glabrata* resistance and susceptibility to *Schistosoma mansoni*. V = Vespiano snail strain; PR = Puerto Rican snail strain; pr = Puerto Rican parasite strain; k = Kenyan parasite strain; R = Resistant-selected snail lines; S = Susceptible-selected snail lines. Each box represents a tank/snail line (new tank for each generation, split into two tanks per line in P_1 and F_3 generations). Each solid arrow represents exposure of all individuals to named parasite strain. Each broken arrow represents the division of snail lines into those to be resistant-selected or susceptible-selected. Each snail tank/line was maintained at matched population sizes within each generation. Heritability of compatibility was investigated following exposure to the same parasite strain across all generations. Strain-specificity of compatibility was investigated following exposure to a novel parasite within the F_3 generation only (see text for further details).

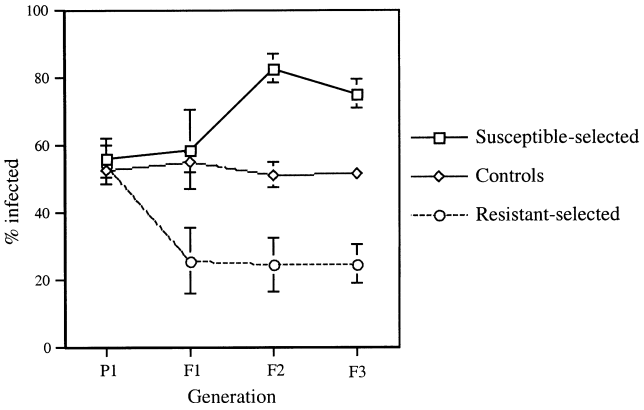

Fig. 2. Heritability of resistance and susceptibility in a *Biomphalaria glabrata–Schistosoma mansoni* system. % infection rate across generations P_1 to F_3 amongst resistant-selected (R), susceptible-selected (S), and unselected control (C) snail lines following exposure to 5 miracidia. Bars represent S.E.M.s across pooled snail–parasite combinations ($n = 4$ replicate combinations per group per generation). Data adapted from Webster & Woolhouse (1998).

was matched, where both were either resistant- or susceptible-selected. In contrast, in the third groups one member of each pair was from a resistant-selected line and the other from a susceptible-selected line. Finally, a group of unselected snails were included to serve as controls. Following an initial period to accommodate for potential sperm storage, snails were left to reproduce, and cross-breeding was identified by Randomly Amplified Polymorphic DNA–PCR (RAPD–PCR; Williams *et al*. 1990). The resulting compatibility phenotype of all offspring was determined.

As would be predicted if resistance were a dominant trait, only the resistance phenotype (75% infection rate) was displayed amongst progeny from either matched resistant-selected pairs and from non-matched pairs where one parent was from a resistant-selected line and the other from a susceptible-selected. The susceptibility phenotype (25% infection rate) was only displayed amongst crosses from matched susceptible-selected pairs. Infection rates, as for the previous Webster &

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Woolhouse (1998) study, remained at approximately 50% amongst unselected control snails exposed to the same parasites (Fig. 1).

These two studies suggest that both resistance and susceptibility to schistosome infection, at least for the two *Bi. glabrata*–*S. mansoni* strain combinations used, are heritable traits, with resistance dominant over susceptibility (Webster & Woolhouse, 1998; Webster, 2001).

Heritability of parasite infectivity and virulence

As for the host resistance described above, one of the best ways to ascertain heritability of virulence and/or infectivity is to document genetic differences in the laboratory.

Hybridisation between closely related schistosome species has been an important tool in demonstrations of the genetic basis of infectivity to a particular snail species or strain. For example, Wright (1974) reported that progeny of a cross between *S. mattheei* and *S. intercalatum* were equally infective to *Bu. globosus* and *Bu. scalarias*, whereas the parental forms were restricted in the case of *S. mattheei* to *Bu. globosus* and in the case of *S. intercalatum* to *Bu. scalaris*. This duel infectivity persisted to the F_3 generation, even though the parasite was passaged solely through *Bu. globosus*; infectivity to *Bu. scalaris* was apparently lost in the F_4 generation. A similar situation occurs with *S. haematobium* and *S. intercalatum* from Cameroon, where parental lines develop only in *Bu. rohlfsi* and *Bu. forskalii* respectively, but their F_1 hybrids can develop in both snail hosts (Rollinson & Southgate, 1987).

Another 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). Arrays of lines are scored for a trait under identical laboratory conditions, and the presence of significant between-line variation is attributed to genetic differences. Several such studies using inbred parasite lines have found evidence of the heritability of infectivity in snail–schistosome systems. For example, Cohen & Eveland (1988) reported consistent differences between clones of *S. mansoni* derived from monomiracidial infections of an inbred laboratory strain and maintained by serial microsurgical transplantation of sporocysts from infected to uninfected *Bi. glabrata*. The infectivity of individual clones in snails ranged from 44 to 100% and were highly consistent within each clone, irrespective of time or subpassage frequency, thereby suggesting that the differences had a genetic basis. Likewise, McManus & Hope (1993) conducted a series of experiments with inbred *S. mansoni* strains originating from different areas and revealed a wide range of infectivities. Moreover, they determined that *S. mansoni* derived from a single geographic population compromise a diverse population with respect to infectivity to snails.

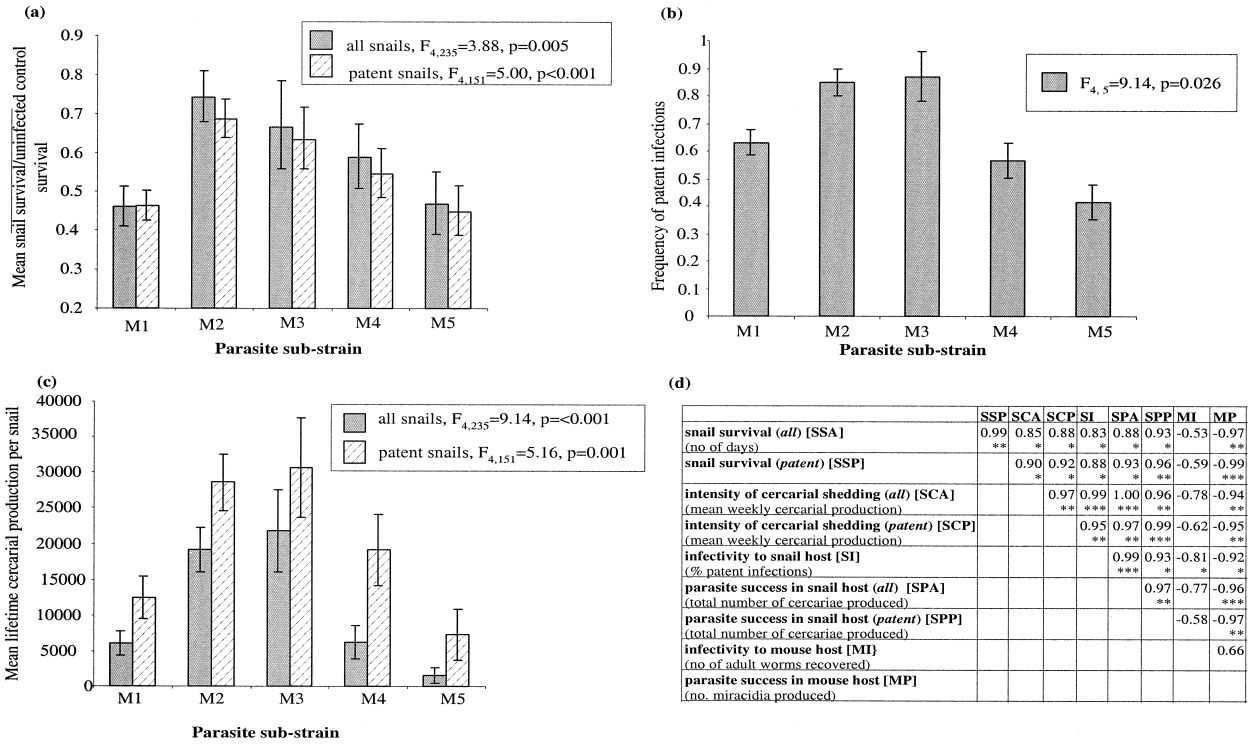
Whilst the aforementioned studies investigated the heritability of infectivity, a recent study by Davies, Webster & Woolhouse (2001) aimed to determine the heritability of both infectivity and, for the first time in the snail host, virulence. Five substrains of *S. mansoni* from a laboratory strain originally from Puerto Rico were developed. In the F_0 generation snails were exposed to a single miracidium and infected snails were randomly paired and used to infect a single mouse host. Since single miracidial infections had been used, cercariae arising from a single snail represented a single clonal population, derived by asexual reproduction. Five egg-producing lines were recovered, arising from crosses between a male and female cercarial clone infection. In two subsequent generations, groups of sexually mature *Bi. glabrata* were individually exposed to *S. mansoni* miracidia from each substrain and cercariae harvested used to infect groups of mice. As shown in Fig. 3a, b, there were significant differences in both infectivity (measured as the frequency of patent infections) and virulence (measured as a reduction in host survival) between substrains. Moreover, such patterns were stable across two generations. This indicated that both these parasite traits have a genetic basis, and that these were heritable over two generations.

In a subsequent study, Davies & Webster (in press) used artificial selection to produce lines differing in virulence to the snail host. Selection was conducted on replicate laboratory strains from two widely differing geographic regions on the intensity of infection, which has been shown to be a correlated factor of virulence (Barbosa, 1975, Davies *et al.* 2001). As shown in Fig. 4, virulence was significantly reduced by artificial selection. This thus provided further support for the heritability of virulence in the snail–schistosome system.

Selective pressures for the evolution of host resistance and parasite infectivity and virulence

The aforementioned studies demonstrate genetic variation and/or heritability in host resistance and susceptibility, as well as parasite infectivity and virulence. However, in order to infer coevolution it still remains necessary to demonstrate that parasite infection and virulence affect host fitness and, conversely, that host resistance affects parasite fitness. Once again there has been some progress in this area using examples from snail–schistosome interactions.

Parasites, by definition, have fitness-reducing effects on their hosts (Ebert & Herre, 1996). Accordingly, schistosomes can be extremely virulent parasites of their intermediate hosts and infection has been reported to cause a number of host fitness-



(d)

	SSP	SCA	SCP	SI	SPA	SPP	MI	MP
snail survival (*all*) [SSA] (no of days)	0.99 **	0.85 *	0.88 *	0.83 *	0.88 *	0.93 *	-0.53	-0.97 **
snail survival (*patent*) [SSP]		0.90 *	0.92 *	0.88 *	0.93 *	0.96 **	-0.59	-0.99 ***
intensity of cercarial shedding (*all*) [SCA] (mean weekly cercarial production)			0.97 **	0.99 ***	1.00 ***	0.96 **	-0.78	-0.94 **
intensity of cercarial shedding (*patent*) [SCP] (mean weekly cercarial production)				0.95 **	0.97 **	0.99 ***	-0.62	-0.95 **
infectivity to snail host [SI] (% patent infections)					0.99 ***	0.93 *	-0.81 *	-0.92 *
parasite success in snail host (*all*) [SPA] (total number of cercariae produced)						0.97 **	-0.77	-0.96 ***
parasite success in snail host (*patent*) [SPP] (total number of cercariae produced)							-0.58	-0.97 **
infectivity to mouse host [MI] (no of adult worms recovered)								0.66
parasite success in mouse host [MP] (no. miracidia produced)								

Fig. 3. Genetic variation for infectivity and virulence in a *S. mansoni* strain and genetic correlations with other fitness traits. Five sub-strains were developed (M1–M5) from a laboratory *S. mansoni* strain originally from Puerto Rico in order to investigate evidence of parasite genetic variation for virulence and infectivity. Sub-strains were developed from the mating of single clones of female schistosomes to single male clones. Groups of snails were exposed to each sub-strain and life-history parameters recorded. Mean values of each parasite sub-strain over two generations were compared by analysis of variance and are shown for (a) virulence (the opposite of host survival), (b) infectivity (the frequency of patent infections) and (c) lifetime cercarial production in the snail host (the number of cercariae produced was recorded weekly for all snails until snail death). Genetic correlations of virulence and infectivity with other fitness traits including parasite reproduction in snail hosts, and infectivity and parasite reproduction in the mouse definitive host, were demonstrated by comparing mean values per parasite sub-strain using Pearson's correlation coefficients. Fitness traits are shown for all snails (*all*), the subset of patently infected snails (*patent*), and mouse definitive hosts (d). Significant correlations are highlighted $^{***}P < 0{\cdot}001$, $^{**}P < 0{\cdot}01$, $^{*}P < 0{\cdot}05$. Data adapted from Davies, Webster & Woolhouse (in press).

reducing effects. For example, Woolhouse (1989), through capture-recapture techniques, reported reductions in survival in naturally infected populations of *Bi. pfeifferi* and *Bu. globosus* in the field, as did Sturrock (1973) for *Bi. glabrata*. Likewise, Pan (1965) and Webster & Woolhouse (1999) showed significantly higher mortality rates amongst infected than uninfected lines of *Bi. glabrata*, and Woolhouse (1989) for *Bi. pfeifferi*, in the laboratory. However, increased mortality rates need not always be direct. For example, schistosome infection has also been shown to reduce the tolerance of infected snails to elevated temperature, molluscicidal chemicals and to heavy metals such as zinc (Bayne & Loker, 1987).

Schistosomes can also affect host fitness through reducing the reproductive success of infected individuals. Reductions of fecundity in molluscan hosts due to infection with schistosomes are observed where the parasite is thought to 'castrate' its host in order to divert resources towards its own development. For example, reductions in the number of egg masses laid by infected snails and/or the number of embryos hatched have been reported in the *S. mansoni–Bi. pfeifferi* (Sturrock. 1966), *S. mansoni–Bi. glabrata* (Sturrock & Sturrock, 1970) and *S. haematobium–Bu. globosus/truncatus/senegalensis* systems (Fryer *et al.* 1990). However, the extent of the inhibition may be linked to the stage of reproductive maturity of the host (Fryer *et al.* 1990), and in several cases fecundity inhibition may be preceded by a short-term burst in egg output (Minchella & LoVerde, 1981; Minchella *et al.* 1985). Other ways in which schistosome infection may affect host reproductive success is through influencing host behaviour. Rupp (1996) studied the mating behaviour of *S. mansoni*-infected and uninfected lines of *Bi. glabrata* and *Bi. alexandrina* in the laboratory. The mating frequencies of patently infected snails were lower than those of controls, which was concluded to result from stress induced by the pathology of infection.

There is less evidence specifically documenting the effects of hosts on parasite fitness. Nevertheless, of the limited data available, *Bi. glabrata* snails

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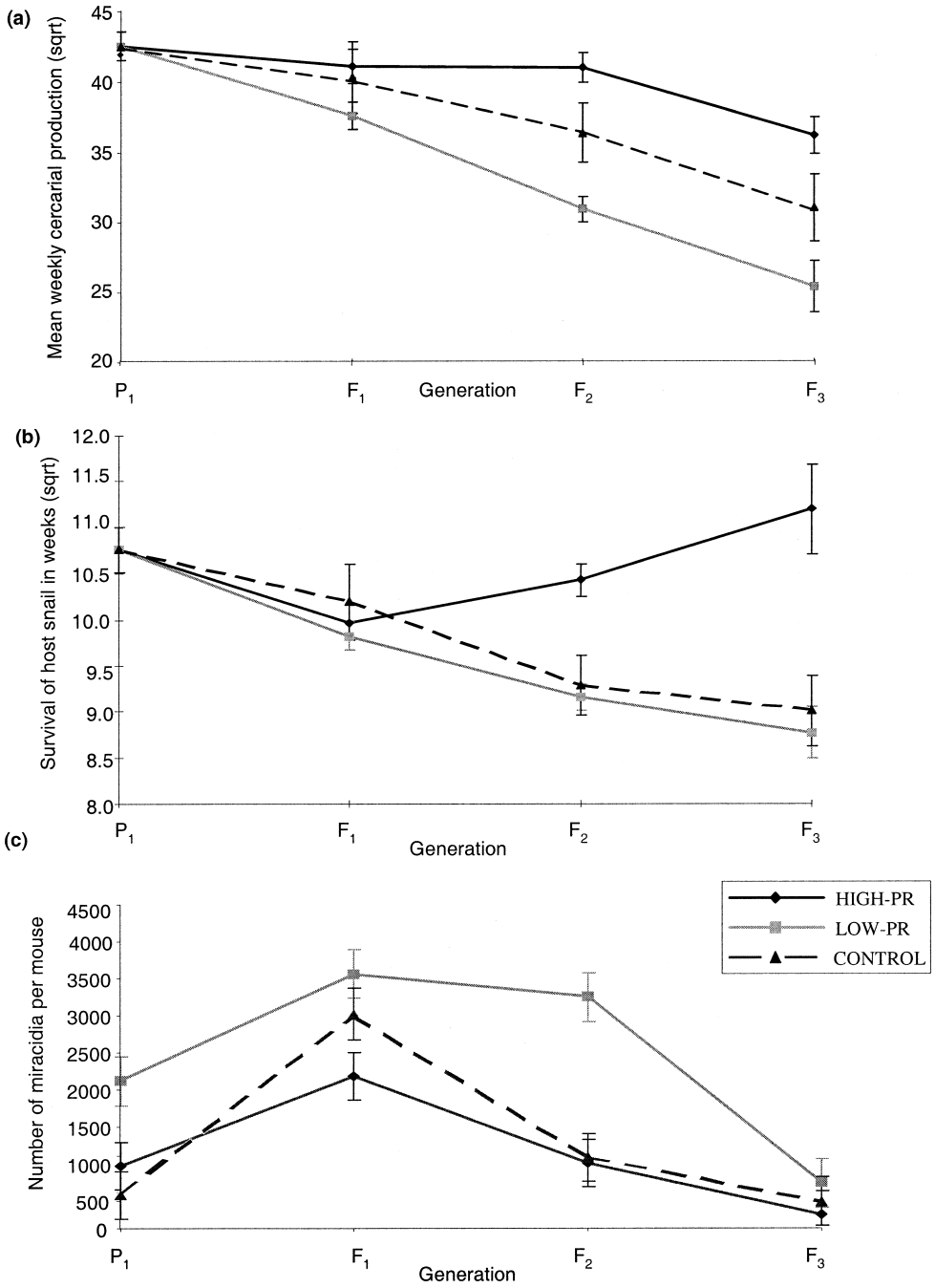

Fig. 4. Heritability of parasite reproduction and virulence in two replicate *S. mansoni* strains. (a) Artificial selection of two laboratory *S. mansoni* strains, one originally from Puerto Rico, and one from Kenya for high and low parasite reproduction (PR) (measured as the number of cercariae produced at week 7 post infection). The mean of two replicates and two unselected control lines are shown. The average weekly cercarial production was significantly increased in HIGH PR-selected and reduced in LOW-PR selected lines ($P < 0{\cdot}001$). There was no overall difference in reproductive rate between the two replicates ($P = 0{\cdot}09$) or in the response of the replicates to artificial selection ($P = 0{\cdot}48$). (b) Snail survival was measured daily. Survival was significantly higher (i.e. virulence was lower) in snails infected with parasites selected for HIGH reproduction than those infected with parasites with a LOW intensity of infection ($P < 0{\cdot}001$). (c) Transmission and success in the definitive host was measured as the number of miracidia present in the livers of mice infected with 220 cercariae at 7 weeks post snail infection. Miracidial production was significantly increased in mice infected with LOW PR-selected parasites and significantly reduced in HIGH-PR selected parasites ($P < 0{\cdot}001$). Data from Davies (2000).

differing slightly in their susceptibility to *S. mansoni* infection have shown dramatic differences in cercarial output per snail (Ward *et al.* 1988), although this was not found by Manning *et al.* (1995) for their *Bu. globosus–S. haematobium/mattheei* combinations. Preliminary work in our laboratory has also shown differences in parasite reproductive success and/or transmissibility depending on the resistance status of

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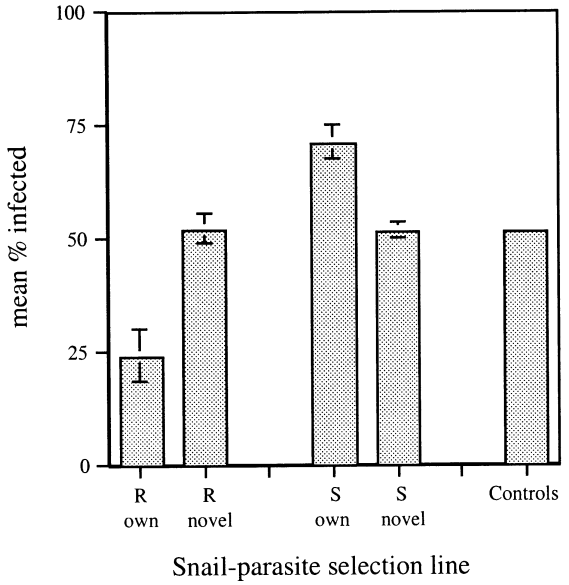

Fig. 5. Strain-specificity of resistance and susceptibility in a *Biomphalaria glabrata–Schistosoma mansoni* system. Following three generations of artificial selection, mean (and SEM) infection rates averaged 75 % amongst resistant-selected snail lines (R) and 25 % amongst susceptible-selected lines (S) following exposure to their own familiar parasite strain (i.e. the parasite strain to which their selection had been based). In contrast, infection rates averaged 50 % amongst unselected control snail lines and also amongst selected snail lines following exposure to a novel parasite strain. Pooled results from four replicate groups (of $n = 60$ snails) per snail line. Data adapted from Webster & Woolhouse (1998).

their intermediate hosts (Blair *et al.* in press; Webster, J. P. unpublished).

ASSESSMENT OF THE APPLICABILITY OF COEVOLUTIONARY MODELS IN SNAIL–SCHISTOSOME INTERACTIONS

The results of the field and laboratory studies described above may suggest snail–schistosome coevolution. What now remains is a consideration of how applicable the currently available data on snail–schistosome interactions are to that of the major coevolutionary models and theories proposed within the first section.

Frequency-dependent or 'Red Queen' coevolution

The specificity of host–parasite interactions is a fundamental assumption of frequency dependent or Red-Queen coevolution (Hamilton, 1980; Hamilton, Axelrod & Tanese, 1990; Lively, 1999). Moreover, the greater compatibility amongst many sympatric host–parasite interactions revealed by the reciprocal cross-infection studies described previously in the second section (Manning *et al.* 1995; Morand *et al.* 1996; Woolhouse & Webster, 2000), suggests such specificity in snail–schistosome interactions. Webster & Woolhouse (1998) thus investigated the potential strain-specificity of snail–schistosome compatibility in the laboratory. The same initial artificial-selection protocol towards resistant-selected and susceptible-selected snail lines, as described above (Fig. 1), was performed on two populations of *Bi. glabrata* snails and two *S. mansoni* parasite populations until the F_2 generation. At the F_3 generation, only half the selected snails from each line were exposed to the same (their 'own') parasite strain to which they had been selected. The other half were exposed to a novel *S. mansoni* strain to which they had not been selected. A matched number of control snails were exposed to both parasite strains. The results suggested that compatibility in this system is strain specific, as the infection rate amongst F_3 snails exposed to a novel parasite was approximately 50 % amongst both resistant- and susceptible-selected snails, and hence matched to that of unselected controls. The resistance (75 % infection rate) and susceptible (25 % infection rates) phenotypes were only observed in snails following exposure to the same single parasite strain to which their artificial selection was focused (Fig. 5).

Webster (2001) then investigated whether it is possible to cross-breed such single strain-specific *Bi. glabrata* snails to be resistant and/or susceptible to more than one *S. mansoni* strain. As for the dominance study described above, individual adult snails from each artificially-selected replicate snail line were paired with a selected partner of matched or non-matched compatibility status. In this case, the first two groups (of $n \geqslant 14$ pairs each) consisted of matched snails in each pair, where each were selected towards the same parasite strain. In contrast, in the third group one member of each pair was of a compatibility status selected towards one (a Kenyan) parasite and the other was selected towards a different (a Puerto Rican) parasite strain. Cross-breeding was identified by RAPD–PCR, and the resulting compatibility phenotype of all offspring was determined. The resistance phenotype (75 % infection rate) was simultaneously displayed against both parasite strains amongst resistant-selected crosses arising from non-matched pairs. The converse was also suggested amongst some susceptible-selected crosses (25 % infection rate). Likewise, as for the previous study (Webster & Woolhouse, 1998), single strain-specificity of compatibility was demonstrated, as all effects were lost if snails were exposed to a novel parasite strain (50 % infection rate, as for unselected controls).

The results from both these studies (Webster & Woolhouse, 1998; Webster, 2001) thus suggest that *Bi. glabrata–S. mansoni* resistance and susceptibility is, as predicted by coevolutionary models such as that of the Red Queen hypothesis, strain-specific. Such strain-specificity may also provide empirical

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support for genetic theories such as the matching allele at multiple loci model of coevolution, which states that each host allele confers resistance to one parasite allele, and parasites may successfully infect a host only when there is an exact match between host and parasite alleles (Frank, 1996). This contrasts with, for example, the 'gene-for-gene' hypothesis, where two alleles at a single locus in both host and parasite are involved (Frank, 1994). Even a system based on a single locus with multiple alleles responsive to selection for graded increases in recognition and affinity to certain parasite-strain characteristics and not others, is unlikely to reflect this snail–schistosome data. Any change in allele frequencies for one effect, such as selection for resistance to one parasite strain, would naturally result in a change in allele frequencies in the remainder, such as for resistance to another strain. No such associations were observed. The results thus support the existence of a multi-locus trait, at least a two-locus, two allele model with the resistance, susceptible, and control lines differing in allele frequencies. Although it is unlikely that there is an allele determining compatibility for every parasite strain, at least some, such as the two examined in the studies above, appear to involve different alleles.

Unfortunately, the potential strain-specificity of parasite infectivity and virulence has not yet been so intensely investigated as has host resistance. However, Richards (1975*b*, *c*) did expose well-characterized strains of *Bi. glabrata* snails to miracidia from strains of *S. mansoni* differing in infectivity, and showed that infectivity was determined by a number of factors with at least one such genetic factor being sex-linked. Similarly, the ability to breed schistosome hybrids infective to the snail strain or species of both parental forms suggests a form of complementation and involvement of multiple infectivity factors. Finally Davies (2000), in an extension of the artificial selection study for reduced *S. mansoni* virulence described above (Fig. 4), demonstrated that the effect was specific to the host strain used in selection. In the F_0 generation, each of two parasite strains were used to infect two groups of inbred laboratory strains of *Bi. glabrata*, one originally from Brazil and one from the Caribbean. One parasite strain was subjected to two-way artificial selection in the Brazilian snail strain, and the other parasite strain in the Caribbean snail strain. Exposure of all selected parasite lines to both snail strains in the F_3 generation demonstrated that the reduced virulence of 'HIGH–PR' selected parasites in the F_3 generation compared to the original F_0 population, and to the F_3 control and 'LOW–PR' lines, was only seen for each replicate in the snail host strain which had been used in selection (i.e. in the Brazilian snail strain for one and the Caribbean strain for the other parasite replicate). There was no evidence of a difference in virulence of the F_3 generation selected parasites and the original F_0 parasites in the snail strain not used for selection (Davies, 2000). Such apparent strain-specificity may suggest that schistosome virulence may also involve a multi-locus trait (Webster & Woolhouse, 1998; Webster, 2001).

Thus strain-specificity has been demonstrated on the sides of both snail and schistosome. This not only suggests that different gene combinations may be involved with each local host–parasite interaction (Rollinson & Southgate, 1987), but it also suggests the potential for Red-Queen coevolution in this system.

However, evidence of Red-Queen dynamics in the field also requires documentation of frequency-dependent selection of host genotypes and a 'rare advantage' (reduced susceptibility of rare hosts). This is a difficult question to answer in sexual populations such as that of *Biomphalaria* and *Bulinus* snails because there are currently no existing markers for the relevant genotypes – i.e. those that are directly involved in the host–parasite interaction. Nevertheless, support is available from another snail–trematode system: *Microphallus* spp.–*P. antipodarum* (Dybdahl & Lively, 1995, 1998, and see Lively, this supplement). Dybdahl & Lively (1995) selected a lake in New Zealand in which all the snails are asexual. In these clonal lines, the genotypes for resistance in the snails are inextricably linked with their multilocal allozyme genotypes. In an initial survey of this lake they discovered four relatively common clones (defined by their allozyme genotypes) and found that the most common clone was significantly over infected (Dybdahl & Lively, 1995). The authors argued that if parasites are driving coevolutionary cycles, they would expect this most-common clone to be driven down in frequency and a different clone would become the most common in the population. Furthermore, they predicted that any changes between years in clone frequency would be correlated with changes in the frequency of infections in that clone at some future point. Support for both of these predictions were provided across a five-year study (Dybadhl & Lively, 1998), and thus the parasites did appear to be driving oscillatory dynamics in the host population. Laboratory experiments confirmed that clones that had been rare in the population for the previous four years were significantly less susceptible than common clones. The combination of results from this study therefore show strong evidence for a rare advantage, as well as evidence for time-lagged selection in the field. Both of these results are again consistent with the Red Queen hypothesis in this system (Lively, 1999).

Cost-benefit trade-offs and locally fixed optima

Cost-benefit trade-offs will be important in host–parasite coevolution, whether locally fixed optima or

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dynamic fluctuating coevolution is expected, as they may have implications regarding, for example, optimal levels observed or the speed of evolution. Trade-offs may also be an important mechanism for the maintenance of genetic variation for resistance, infectivity and virulence in natural populations. Trade-offs may take many forms, although empirical evidence is often lacking. Nevertheless, once again, some of the few animal host–parasite examples come from snail–schistosome interactions.

Costs of resistance. It has frequently been argued that for there to be a stable genetic polymorphism in disease resistance the fitness of the resistant genotypes should be less than that of the susceptible genotypes in the absence of the disease. The argument is intuitively obvious: without such a cost, an allele for resistance should continue to increase in frequency as long as some disease is present (Antonovics & Thrall, 1994). Accordingly, there is no evidence for a fixation of resistance amongst wild snail populations (Manning *et al*. 1995). Webster & Woolhouse (1999) investigated potential costs of resistance in a *Bi. glabrata–S. mansoni* host–parasite system. Once again, using artificial selection to breed snails that are resistant or susceptible to schistosome infection, their study investigated whether compatibility has any associated cost in terms of snail fertility (defined as actual reproductive performance, measured as the number of offspring produced) and/or fecundity (defined as potential reproductive capacity, measured as number of eggs and embryos formed). Indeed, susceptible-selected snail lines showed significantly higher fertility than resistant-selected or unselected control snail lines, irrespective of current infection status. In contrast, there were no significant differences between snail lines in fecundity, proportion of abnormal egg masses produced, or mean number of eggs per egg mass. These results are consistent with snails incurring costs of resistance to schistosome infection in the absence of the parasite.

Similar results were also found by Cooper *et al*. (1994), where again fertility rates were significantly lower amongst resistance-selected snails lines. However, Cousin *et al*. (1995) showed that significant abnormalities exist in the snail strain used by Cooper *et al*. (1994) which were suspected to have resulted from the intense inbreeding of this stock. Thus the direct link between fertility and resistance proposed by Cooper *et al*. (1994) remains to be ascertained (Cousin *et al*. 1995).

Finally, working on the *Bi. glabrata–Echinostoma caproni* system, Langand *et al*. (1998) also found an apparent cost of resistance, manifested as a delay in reproductive maturity. Again utilising snail lines artificially selected towards resistance or susceptibility, they found that (although only analysing offspring from a single snail pair) that resistant-selected individuals reached maturity approximately four days later than did susceptible snails.

Costs of infectivity. It is also frequently assumed that infectivity alleles must have a negative effect on parasite fitness that offset the benefit of wider host range. This assumption is again necessary because without a fitness cost, the infectivity allele would spread to fixation (Frank, 1993), and polymorphisms would not be maintained. Costs will also be important in determining the optimal level of infection in certain situations. Davies *et al*. (2001) determined that though there was an overall cost of infection in the snail–schistosome system utilized, manifested as a reduction in host, and hence parasite, survival, higher levels of infectivity were not apparently associated with increasing fitness costs to the host (and hence indirectly to the parasite). In fact, as shown in Fig. 3d, infectivity was positively associated with both snail survival and cercarial shedding intensity. This resulted in significant differences in parasite fitness (measured as the lifetime production of cercarial stages) between inbred lines (Fig. 3c). Similar patterns of positive associations of cercarial shedding intensity, infectivity and survival have been previously reported by Barbosa (1975), who examined 16 strains of *Bi. glabrata* and 233 strains of *Bi. straminea*.

Davies *et al*. (2001) further found that there was a parasite fitness cost of increased infectivity to the snail host due to a trade-off in infectivity between the different hosts of the schistosome lifecycle (see Fig. 3d). Overall, infectivity to and cercarial production in the snail host were negatively correlated with infectivity to and miracidial production in a mouse definitive host. This unexpected result of a trade-off in fitness between the hosts of the schistosome lifecycle was substantiated in the artificial selection experiment described in Fig. 5. This could therefore explain the presence of polymorphisms in infectivity within such a laboratory *S. mansoni* strain.

Benefits of virulence. Virulence reduces parasite fitness by reducing the duration of infection. Fitness costs of being virulent have been documented in the snail–schistosome system by the reduction in total cercarial production in highly virulent parasite strains (i.e. where snail survival is lower) (Davies *et al*. 2001: Fig. 3). Current theory suggests that virulence may be maintained and promoted where the parasite faces a trade-off between traits which are correlated with fitness, in particular that the fitness costs of high virulence may be offset by the benefits of increased transmission or ability to withstand host defences (Frank, 1996). The evolution of stable virulent parasites thus requires parasite factors that are genetically correlated. A genetic correlation results in linkage between traits that restricts their evolution (Ebert & Herre, 1996) such that, for

example, transmission cannot be increased without further increasing virulence. Many evolutionary models propose that virulence is maintained as a side-effect of parasite fecundity, for example if a high concentration of parasites in a host increases the probability of transmission to new hosts or decreases the probability of host recovery, but also increases host death rate (Bull, 1994). In this instance, natural selection is expected to optimise parasite reproductive rates such that the number of new infections is maximized. Davies *et al*. (2001; Fig. 3c) and Davies & Webster (in press; Fig. 4) suggest that virulence in the snail host may be maintained by increased transmission to the definitive host. However, as previously mentioned, variations in reproductive rate in the snail host is not thought to be the mechanism involved.

Multiple infections are a further mechanism that have been proposed to promote the evolution of increased parasite virulence, since intra-host competition can favour high virulence parasite strains of lower potential fitness than less virulent strains wherever they have a local growth or transmission advantage (e.g. Frank, 1996; May & Nowak, 1995; van Baalen & Sabelis, 1995; Nowak & May, 1994; Bonhoeffer & Nowak, 1994; Anita, Levin & May, 1994; Levin & Pimental, 1981). Multiple infections of single snail hosts by more than one schistosome genotype have been detected in both *Bi. glabrata* (Minchella *et al*. 1995) and in *Bu. globulus* (Davies *et al*. 1999; Woolhouse, Chandiwana & Bradley 1990). Preliminary investigations in our laboratory have also suggested the potential for competitive interactions to occur between schistosome strains within snail hosts (Davies, 2000). Demonstration of competition requires evidence that *per capita* parasite success is reduced by the presence of the other parasite line. Groups of snails were therefore exposed to one or other, or a combination of two of the parasite strains developed in Fig. 3. A genetic marker was used to identify resulting progeny in order to estimate the success of each parasite line. The success of parasite M1 sub-strain was shown to be reduced in the presence of the faster growing parasite genotype, M2. This was true irrespective of the relative proportion of M1 sub-strain in the original dose. The success of M2 sub-strain, however, was not affected by the presence of the other line. This study therefore demonstrated evidence of competition between parasite genotypes and asymmetry of competitive interactions (Davies, 2000).

Benefits of resistance. Apart from the obvious benefits of host resistance, in terms of reduced infection and parasite-induced mortality etc. rates, further benefits of resistance may be necessary to maintain resistance genes in the absence of infection. In accordance, potential preferential mate choice towards uninfected (Rupp, 1996), and even resistant-selected snails, irrespective of their infection status (Webster, 2001 and unpublished data), has been identified in this system, which could account for the maintenance of resistance polymorphisms even amongst unselected laboratory snail stocks.

Therefore, these data serve to demonstrate the potential for costs and benefits of resistance, virulence and infectivity, each of which may affect both the coevolutionary process itself, and the related question regarding the maintenance of polymorphism.

The geographic mosaic theory of coevolution

Subdivision of populations, the spatial pattern of selection, migration and gene flow in hosts and parasites can all influence the coevolutionary process (Dybdahl & Lively, 1996). Such patterns may determine whether or not contact between a host and parasite species will lead to the establishment of a long-term interaction and also the geographic scale over which any such interactions will occur (Burdon & Thrall, 1999). In a number of population genetic surveys (reviewed by Jarne & Theron, this supplement), significant spatial sub-structuring of both snail and schistosome populations has been reported. However, such spatial sub-structuring may vary both within and between seasonal collections, as has recently been shown for *Bi. pfeifferi* in the Zimbabwean highveld (Hoffman *et al*. 1998; Webster *et al*. 2001; Davies *et al*. 2001). Abiotic factors, in particular rainfall and river type, have also been inferred to influence population stability (Webster *et al*. 2001; Kruger *et al*. 2001). Thus differential selection in host–parasite interactions may be expected in different populations of the *Bi. pfeifferi* metapopulation, as would be predicted by the geographic mosaic model of host–parasite coevolution (Thompson, 1994).

Thus in summary, the results of the studies reviewed above suggest that snail–schistosome interactions can provide much needed empirical support for several of the major coevolutionary models and theories, in particular that of the Red Queen hypothesis, Cost–Benefit trade-offs, and the Geographic Mosaic Theory.

CONCLUSIONS AND AREAS FOR FURTHER STUDY

The results of the numerous studies reviewed here have both added a great deal to our understanding of the system and served to highlight the valuable role snail–schistosome interactions may play in exploring general issues of the coevolution of resistance, infectivity and virulence. This may be particularly pertinent as this is an indirectly transmitted macroparasite system, so often overlooked amongst both theoretical and empirical studies.

However, as is always the case in science, the

results produced so far raise as many questions and areas for subsequent research as they answer. For example, in terms of snail–schistosome coevolution and compatibility, cross-species interactions have not yet been considered. In many geographic areas, particular snail species are infected by more than one schistosome species, such as the infection of *Bu. globosus* with human *S. haematobium* and bovine *S. mattheei* in Zimbabwe (Manning *et al*. 1995). Yet, there is currently no information as to whether selection for improved resistance against one species of parasite is associated with improved or reduced defence against other species. Likewise, much remains to be investigated into other potential fitness costs, such as the susceptibility to predation of resistant snails or the fitness costs of being exposed but not infected. Long-term controlled laboratory experiments and field surveys aimed to document coevolution should be developed. Measurements of levels of resistance, infectivity and virulence in natural populations and the existence of polymorphism under differing epidemiological situations is also obviously an important next step in understanding snail–schistosome coevolution. DNA probes developed from molecular markers linked to resistance, infectivity and virulence genes would facilitate such studies. Knight *et al*. (1999) were recently successful in identifying resistance markers, although the population genetic variability and strain-specificity of compatibility documented here (Jarne & Theron, this supplement, Webster *et al*. 2001; Davies *et al*. 1999; Hoffman *et al*. 1998) may suggest that the applicability of such probes to the field would be severely limited. The effort to clarify the role of genetic factors involved in snail–schistosome compatibility is challenging in many respects. Both participants are complex metazoans with numerous chromosomes and relatively large genome size. Furthermore, factors controlling compatibility vary within and between populations and with host age (Bayne & Loker, 1985). One of the greatest challenges will therefore be to integrate genetics, evolutionary biology and population dynamics into models of resistance, infectivity and virulence that can generate hypotheses that can be tested in the field and laboratory on snail–schistosome and other host–parasite systems.

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