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Are *Biomphalaria* snails resistant to *Schistosoma mansoni*?

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Abstract

Among *Biomphalaria glabrata/Schistosoma mansoni* snail–trematode combinations, it appears that some parasites succeed whilst others fail to infect snails. Snails that become infected are termed susceptible hosts. Those which are not infected are traditionally determined as 'resistant'. Here the concept of *B. glabrata* resistance to *S. mansoni* is re-examined in the light of additional observations. It is suggested that, in *B. glabrata/S. mansoni*, compatibility is tested independently for each individual miracidium and host, and that the success or failure of an infection does not depend on the snail susceptibility/resistance status, but on the 'matched' or 'mismatched' status of the host and parasite phenotypes.

Introduction

It is largely admitted that the success or failure of host–parasite infections results from a complex interplay between host defence mechanisms and parasite infectivity strategies. However, within a compatible snail–trematode combination, it is generally considered that an unsuccessful infection reflects the existence of snail resistance processes, whereas a successful infection reveals the susceptible status of the snail host. Here the term resistance refers to the 'inherent ability of organisms to prevent establishment or development of a given species or strain of compatible parasite' (Coustau & Théron, 2004).

This concept of susceptibility/resistance of snails towards trematode infections seems appropriate to most immunosuppressive trematodes. In such systems as *Biomphalaria glabrata/Echinostoma* sp. or *Pseudosuccinea columella/Fasciola hepatica*, trematodes actively interfere with the defence response of the susceptible snail host, while resistant hosts remain unaffected by this immunosuppressive effect (DeGaffé & Loker, 1998; Humbert & Coustau, 2001; Gutiérrez *et al.*, 2003).

In the present paper, the question of the existence of resistance processes in the *Biomphalaria glabrata/Schistosoma mansoni* association is addressed. The debate is not new, since Wright (1974), using snail/schistosome examples, raised the question of 'Snail susceptibility or trematode infectivity?' decades ago. However, most recent studies have focused on the functional aspects or genetic basis of resistance, using laboratory-selected strains, and the concept of snail resistance is now generally accepted. As the existence of host resistance processes has several functional and evolutionary implications (Webster & Woolhouse, 1999; Webster & Davies, 2001; see however Coustau *et al.*, 2000; Rigby *et al.*, 2002), it is now time to re-examine the concept of *B. glabrata* resistance in the light of additional observations.

B. glabrata/*S. mansoni* compatibility: a highly relative concept

Compatibility between isolates or strains of snails and schistosomes is generally determined experimentally by infection rates obtained from a number of snails individually exposed to a fixed number of schistosome miracidia. However, it is well established that experimental snail prevalences are dependent on numerous factors particularly the number of miracidia/snails used (Théron *et al.*, 1997). Figure 1 gives an example of two different *B. glabrata/S. mansoni* combinations showing significantly different levels of susceptibility/resistance for a dose of 10 miracidia per snail (30% and 62% of susceptibility respectively). However, when snails are exposed to 30 miracidia, both strains appeared 65% susceptible (unpublished data). In such a case, can we consider that one strain is more susceptible/resistant than the other?

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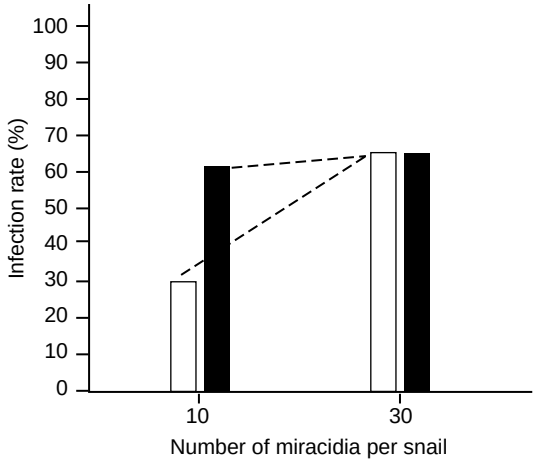

Fig. 1. Infection rates of *Biomphalaria glabrata* from Guadeloupe (Bg MAR and Bg DFO) individually exposed to 10 and 30 miracidia per snail of two strains of *Schistosoma mansoni* from Guadeloupe (SmGH2 and SmDFO, respectively). ■, Sm GH2/Bg MAR; □, Sm DFO/Bg DFO.

The same ambiguity exists at the individual level. Histological sections of *B. glabrata* snails exposed to several miracidia of *S. mansoni* show normally developing mother sporocysts attesting a successful infection (fig. 2A). From this observation one may classify the snail as a susceptible host. However, within the same tissues of this 'susceptible host' we may also observe encapsulated parasites, together with developed mother sporocysts. This means that, according to Basch (1975), compatibility is tested independently for each individual miracidium and that the status of 'resistant host' appears non-adapted in such a case. An other interpretation would be to consider that the success or failure of a snail infection (i.e. parasite surviving vs. parasite encapsulation) results mainly from the ability or inability of a given miracidium to avoid the innate immune system of the host it enters. Then the host defence reaction against uninfective miracidia may not be seen as a specific resistance against *S. mansoni* but as a general defence response against an invasion by living material recognized as non-self.

Infectivity strategy of schistosome miracidia

Even if the nature of the genes and products involved in the *B. glabrata*/*S. mansoni* interaction remains largely unknown, most authors seem to agree with the hypothesis that schistosome sporocysts may escape haemocyte response by expressing epitopes (likely glycoproteins) that mimic host molecules and therefore prevent parasite recognition (Adema & Loker, 1997). This schistosome infectivity strategy, based on molecular mimicry, differs from other trematodes (e.g. echinostomes) which probably are recognized as non-self but have the ability to actively interfere with the defence system of the snail (Loker & Adema, 1995).

Compatibility between *B. glabrata* and *S. mansoni* could then be defined as a concordance between host and parasite molecules, involved in recognition and mimicry mechanisms, respectively. A schistosome miracidium would be infective/uninfective if its genetically determined phenotype is respectively concordant/misconcordant with the host phenotype (Basch, 1975). Such a mechanism is compatible with a matching phenotype model and the existence of a polymorphism of compatibility.

Experimental selected snail resistance: use and limitations

Compatibility polymorphism in *Biomphalaria*–schistosome has been used to establish laboratory colonies of snails selected as 'resistant' or 'susceptible' towards a schistosome strain. These laboratory-selected snail strains are routinely used to investigate host defence/or resistance mechanisms (Bayne *et al.*, 2001; Lewis *et al.*, 2001; Lockyer *et al.*, 2004), characterization of genetic markers associated with susceptibility/resistance (Jones *et al.*, 2001) and ultimately, 'genes of resistance' (Knight *et al.*, 2000). However, a word of warning appears necessary here regarding the status of a 'resistant strain' if compatibility is considered as the result of matched/mismatched phenotypes between host and parasite.

Laboratory strains of *B. glabrata* usually show on average 50% susceptibility towards a sympatric strain of *S. mansoni* (Webster & Woolhouse, 1998) and this level of susceptibility is more or less maintained through generations under standard conditions of snail and mouse infections. From such strains, snail lines with higher levels of susceptibility or higher levels of resistance can be selected within a few generations (Webster & Woolhouse, 1998).

Based on the matching phenotype hypothesis, 50% susceptibility within a control snail strain would mean that the parasite strain contains a variety of phenotypes which match only half of the snail phenotypes present within the host strain (fig. 3A). Selection for susceptibility would consist of eliminating snail phenotypes which do not match the existing parasite phenotypes; a process that increases the infection rate (fig. 3B).

Conversely, selection of 'resistance' would consist in eliminating most of the snail phenotypes that match existing parasite phenotypes and in maintaining snail phenotypes that do not match them (fig. 3C). Such selected snails are not exactly resistant to *S. mansoni* but 'mismatched' with the limited number of parasite phenotypes within the laboratory-cycled parasite strain. Note that this limited number of parasite phenotypes is not representative of the total genetic diversity existing in the original population of the parasite or in other parasite strains (Stohler *et al.*, 2004). In support of this explanation, it has been shown that these snail lines selected as 'resistant' could be susceptible to another strain (Webster & Woolhouse, 1998) or to a fresh isolate randomly collected from the same, but genetically diversified field population of parasite (fig. 3D).

These laboratory selected strains clearly represent valuable tools for elucidating innate immune responses or functional differences between snails displaying resistant or susceptible phenotypes towards particular

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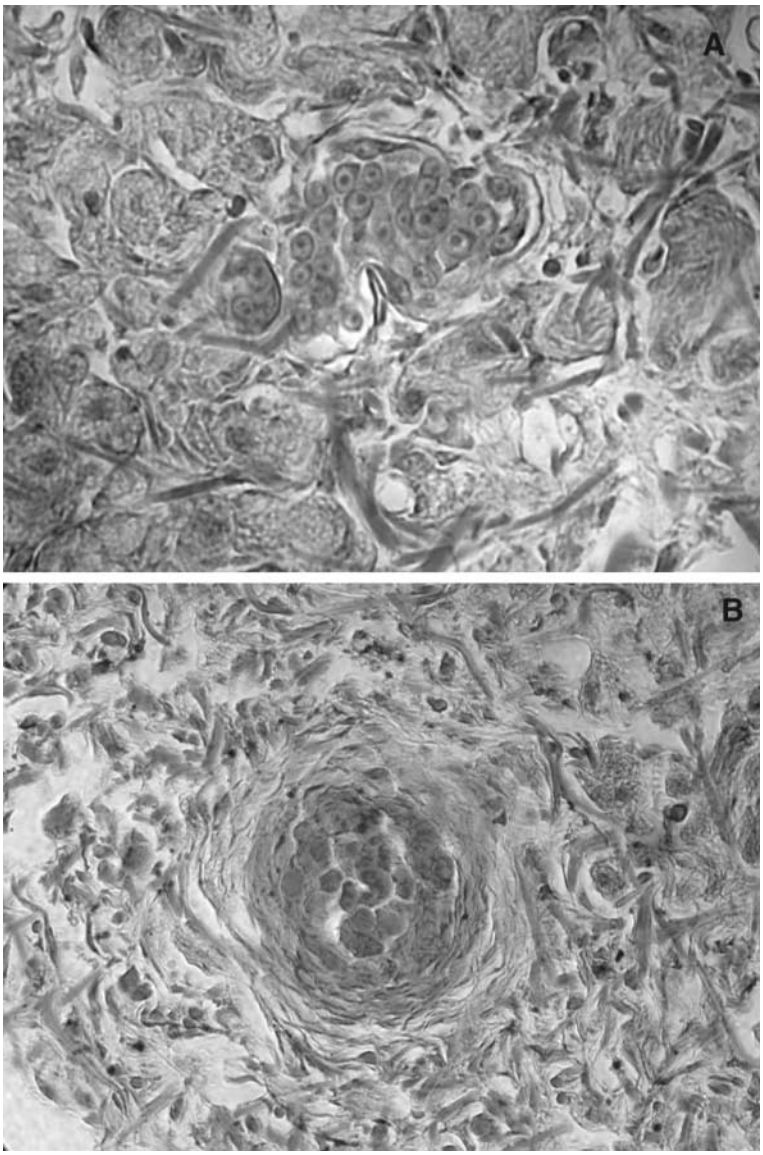

Fig. 2. Developed (A) and encapsulated (B) mother sporocysts of *Schistosoma mansoni* within the same individual of *Biomphalaria glabrata* 48 h after exposure to several miracidia.

schistosome strains. However, if the matched/mismatched phenotype hypothesis is correct, the use of these strains may be misleading for investigating the genetic basis of resistance or for testing evolutionary hypotheses relying on resistance processes (e.g. occurrence of a fitness cost associated with resistance and its role in the maintenance of susceptibility polymorphisms in natural populations).

Concluding remarks

Considering the hypothesis that the success or failure of a *B. glabrata*/*S. mansoni* infection does not depend on the snail susceptibility or resistance status, but depends on the 'matched' or 'mismatched' status of the host and parasite phenotypes, this has several conceptual and practical implications. For example, under this hypothesis, functional studies would have to face the major challenge of identifying both host and parasite factors responsible for the matched, or unmatched status of the host–parasite combination. Particular efforts should be made to identify the molecular determinant of self/non-self recognition and their potential variability. Interestingly, recent studies have demonstrated that the previously characterized family of *Biomphalaria* fibrinogen related proteins (FREPs) undergo processes of recombinatorial diversification leading to the concomitant existence of a great diversity of FREPs within a single individual (Zhang *et al.*, 2004). Although their function has not yet been clarified,

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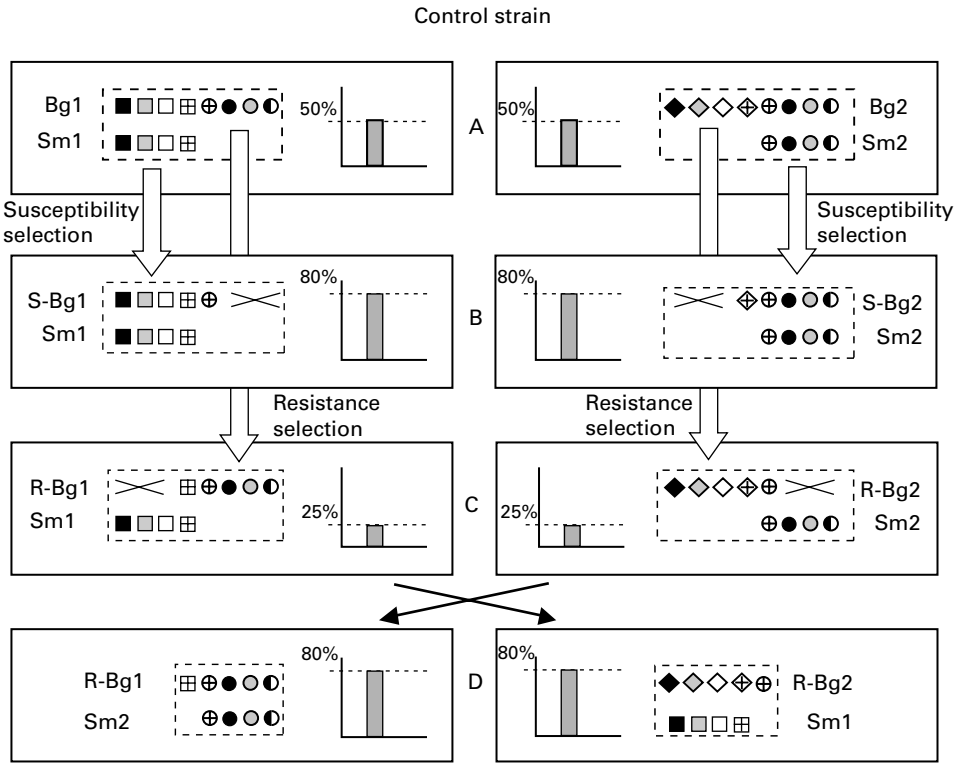

Fig. 3. Schematic representation for the selection of susceptible and resistant lines of snails from two strains of *Biomphalaria glabrata* (Bg1 and Bg2) and *Schistosoma mansoni* (Sm1 and Sm2) under the hypothesis of a matching genotype model (see text for explanation of parts A, B, C and D). Symbols represent genotype diversity for compatibility between snails and parasites.

evidence suggest that FREPs are capable of binding molecules of foreign origin such as *Echinostoma paraensei* excretory–secretory products (Adema *et al.*, 1997), or *S. mansoni* surface epitopes (Hertel *et al.*, 2005). The confrontation of a number of host carbohydrate-binding molecules such as FREPs, to their *S. mansoni* carbohydrate ligands could determine the 'matched' or 'mismatched' status of a *B. glabrata*–*S. mansoni* combination. It is therefore crucial to maintain the current efforts in identifying host carbohydrate-binding molecules as well as schistosome surface glycoconjugates (Castillo & Yoshino, 2002; Nyame *et al.*, 2002).

Finally, a promising approach in the near future may be to associate population and functional studies. In particular, polymorphisms of natural populations could be used in functional studies to compare candidate host and parasite factors in several successful and unsuccessful combinations.

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