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Parasite infection, host resistance and mate choice: battle of the genders in a simultaneous hermaphrodite

Joanne P. Webster[1*], **Joseph I. Hoffman**[1†] **and Manuel Berdoy**[2]

[1]*The Peter Medawar Building for Pathogen Research, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3SY, UK*
[2]*Oxford University Veterinary Services, Parks Road, Oxford OX1 3PT, UK*

Parasites have been proposed to be fundamental in the evolution of mate choice because differential mating on the basis of heritable disease resistance is expected to lead to progeny with a better genome–environment match than random mating. However, direct empirical data in support of this hypothesis are often lacking, and the relative influences of current and potential infection status (i.e. resistance genotype), and of mate choice versus mate conflict, remain largely unknown. We demonstrate experimentally, using simultaneous hermaphroditic snails (*Biomphalaria glabrata*) artificially selected for resistance or susceptibility to *Schistosoma mansoni* infection, that mate choice is influenced by a combination of current and potential parasitic infection status. As predicted by game-theory models, we also found a picture of conflict and cooperation: resistant and susceptible genotypes copulated in either gender and reciprocated (i.e. switched gender) equally when faced with an uninfected partner, but, by contrast, resistant snails actively refused to copulate as females with an infected partner. Such recognition and discrimination has implications for the maintenance of sex and the evolution of recognition systems.

Keywords: mate choice; parasite; host resistance; game theory; snail; schistosomes

1. INTRODUCTION

The landmark paper of Hamilton & Zuck (1982) alerted many biologists to the possibility that parasites were of fundamental importance in the evolution and maintenance of active mate choice (Bateson 1983; Hamilton *et al.* 1990; Read 1990; Jennions & Petrie 1997), since differential mating on the basis of heritable disease resistance is expected to lead to progeny with a better genome–environment match than random mating. However, direct empirical data in support of this hypothesis are often lacking, and the relative influences of current and potential infection status (i.e. resistance genotype), and of mate choice versus mate conflict, remain largely unknown (Bateson 1983; Hamilton *et al.* 1990; Read 1990; Jennions & Petrie 1997). *Biomphalaria glabrata* are simultaneous hermaphroditic freshwater snails that act as intermediate hosts for the macroparasitic trematode *Schistosoma mansoni,* the causative agent of schistosomiasis. Schistosomiasis ranks second only to malaria in terms of parasite-induced human morbidity and mortality, with over 200 million people infected. Snail susceptibility and resistance to schistosome infection have both been shown to have a heritable and strain-specific basis, indicative of matching alleles at multiple loci (Richards *et al.* 1992; Webster & Woolhouse 1998; Webster 2001). Genetic variability for both susceptibility and resistance is maintained within natural snail metapopulations through, in part, trade-offs between the high fitness costs resulting from parasitism and those associated with resistance: susceptible snails suffer from significantly increased mortality when infected, whereas resistant individuals suffer reduced egg viability (despite equal egg mass and embryo production, significantly fewer offspring hatch from resistant lines, potentially owing to deficiencies in internal factors that, although primarily associated with host reproduction, are also essential for normal parasite development; Woolhouse 1989; Cooper *et al.* 1994; Cousin *et al.* 1995; Webster & Woolhouse 1999). Laboratorial behavioural and molecular studies have revealed that *B. glabrata* preferentially outcross (Vernon *et al.* 1995; Vernon & Taylor 1996; Webster 2001). Although data are limited, some evidence of differential mating has been detected among unselected and uninfected *B. glabrata* lines, relating to inbreeding avoidance (Vernon *et al.* 1995; Vernon & Taylor 1996) and geographical origin (Rupp & Woolhouse 1999). No gender biases, where individuals preferentially mate as male or female, were reported (Vernon *et al.* 1995; Vernon & Taylor 1996; Trigwell *et al.* 1997; Rupp & Woolhouse 1999). We investigated here whether an interplay of potential infection status (host resistance versus susceptibility genotypes) and current infection status (infected versus uninfected) influenced the reproductive strategy of *B. glabrata* snails. We observed the mating behaviour of free-moving unselected *B. glabrata* snails, currently infected or uninfected, with partners artificially selected for either resistance (referred to here as 'resistant' snails) or susceptibility (referred to here as 'susceptible' snails) to *S. mansoni* infection.

2. MATERIAL AND METHODS

Artificial selection, carefully controlling for any potential maternal effects, inbreeding or age or size bias, was used to breed *B. glabrata* snail lines that were resistant-selected, susceptible-selected or unselected toward *S. mansoni* parasitic infection (figure 1), as has been described in detail elsewhere (Webster & Woolhouse 1998, 1999; Webster 2001).

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[*] Author for correspondence (joanne.webster@medawar.oxford.ac.uk).
[†] Present address: Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

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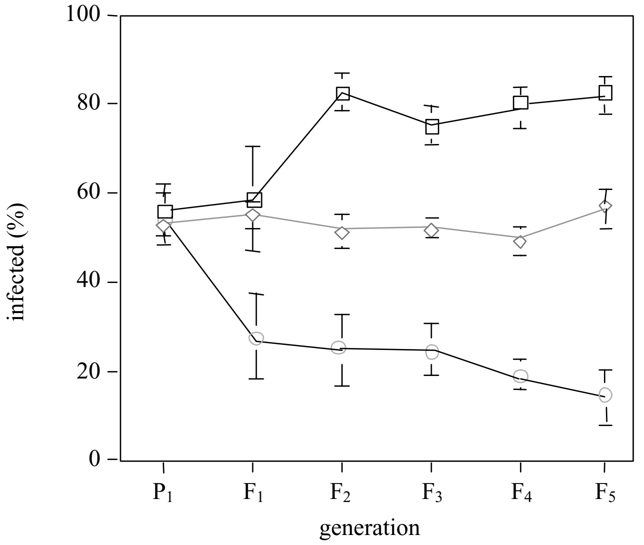

Figure 1. Heritability of resistance and susceptibility to *S. mansoni* infection. Artificial selection, carefully controlling for any potential inbreeding or maternal-effect bias, was used to breed *B. glabrata* snail lines that were resistant-selected, susceptible-selected or unselected toward *S. mansoni* infection. There were highly significant differences between snail lines in the percentage infection prevalence following exposure to five miracidia in the F_1 generation (log-linear analysis: $\chi^2 = 93.83$, d.f. = 2, $p = 0.001$) and this difference remained significant in all subsequent generations (F_2–F_5). Genetic variability, as determined using the randomly amplified polymorphic DNA–PCR technique (see Webster (2001) for details), remained approximately equal between the resistant, susceptible and unselected lines throughout selection (e.g. pairwise Jaccard's similarity coefficients on F_5 snails: Kruskal–Wallis $H = 4.23$, d.f. = 2, $p = 0.11$). Bars represent s.e.m. across pooled snail–parasite combinations ($n = 4$ replicate combinations per group per generation). Squares, susceptible; diamonds, control; circles, resistant. P_1, parent generation.

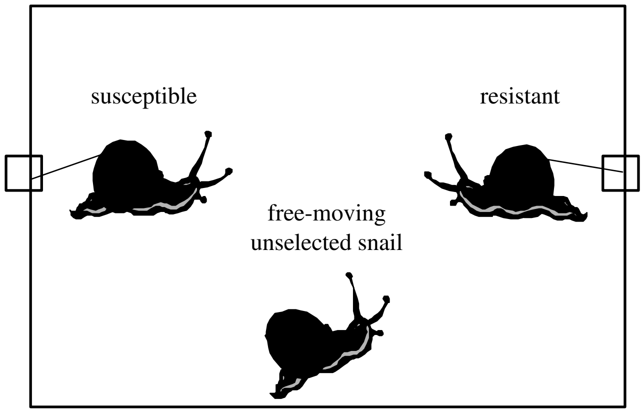

Figure 2. Mating arena. To provide snails with a choice of mate genotype, arenas were set up, each containing three sexually mature *B. glabrata* snails: one free-moving snail from an unselected control line and two tethered snails, one from a resistant-selected line and the other from a susceptible-selected line (see § 2 for details). Analyses were performed upon 19 triplets containing an uninfected free-moving snail and 10 triplets containing an *S. mansoni*-infected free-moving snail, using the same parasitic strain that the tethered snails had been selected toward. All tethered snails were uninfected.

For behavioural analyses, F_5 snails to be observed were removed from their stock lines four weeks before each run and kept in isolation to avoid potential bias in allosperm and autosperm storage (Paraense 1956). Snails were then placed in plastic rectangular 'mating arenas' (4 cm × 6 cm) filled with water, but without food. Each arena contained three sexually mature snails: one free-moving snail from an unselected line and two tethered snails, one from a resistant-selected line and the other from a susceptible-selected line. The tethered snails were restricted to opposite ends of the arena by cotton thread that allowed free movement but prevented them from coming into contact with each other (figure 2). The position of tethered snails within the arena was randomized between replicates. The unselected snail was able to move freely throughout the arena and to copulate with either of the tethered snails (but not both simultaneously). This design avoided the potential bias in mate choice caused by inbreeding avoidance that could occur if, for example, snails had the opportunity to mate with a partner from their own line. The free-moving snails were all from the same unselected stock line and differed only in being either uninfected or infected with the same parasite strain that the tethered snails had been selected toward. All tethered snails were unexposed and uninfected, thereby controlling for any potential bias caused by parasitic 'memory' history. All snails were unknown to each other at the study onset. All snails within each triplet, and across infection groups, were size matched to the nearest 0.05 mm and 0.001 g to prevent any size-advantage gender bias (Ghiselin 1969).

Snail behaviour throughout each continuous 8 hour run (1700–0100) was monitored by direct observation and simultaneously filmed by a close-up video camera for subsequent confirmatory analyses. Eight W fluorescent tubes, placed 10 cm away along each length of the arena (i.e. two tubes per arena) provided constant low-level non-directional illumination. The selection status of each of the tethered snails was unknown to the observers at the time of recording. The position (side of arena), time of initiation, duration and identity of snail initiating and/or terminating each interaction were individually recorded, producing a total of 13 600 lines of data. Mating duration was recorded as the duration that a snail's penis was visibly inserted into the other snail's genital pore (Vernon 1993; Vernon & Taylor 1996; Trigwell *et al.* 1997). Nineteen triplets containing uninfected free-moving snails and 10 triplets containing patently infected free-moving snails were analysed (proc GLM in SAS/STAT v. 81999) using infection status, genotype and gender as the independent variables. All residuals were checked for normality and homogeneity of variance, and the data were transformed when appropriate. Non-parametric (Wilcoxon–Mann–Whitney) tests were carried out if normality was not achieved after transformation and the results are expressed with the p-value calculated from the exact distribution from S (lowest sum of scores for unpaired test, sum of ranks less than 0 for paired tests) or using Lehmann's normal approximation when ties existed in the data.

3. RESULTS

Infection status did not affect the mating intensity of the free-moving snails (mean ± s.e.m. total duration of copulation in minutes per 8 hour night: uninfected 104 ± 13; infected 116 ± 14; $S = 172$, $p = 0.31$). Likewise, free-moving snails mated with the tethered resistant and susceptible snails in equal measure (infected: $S = 103$,

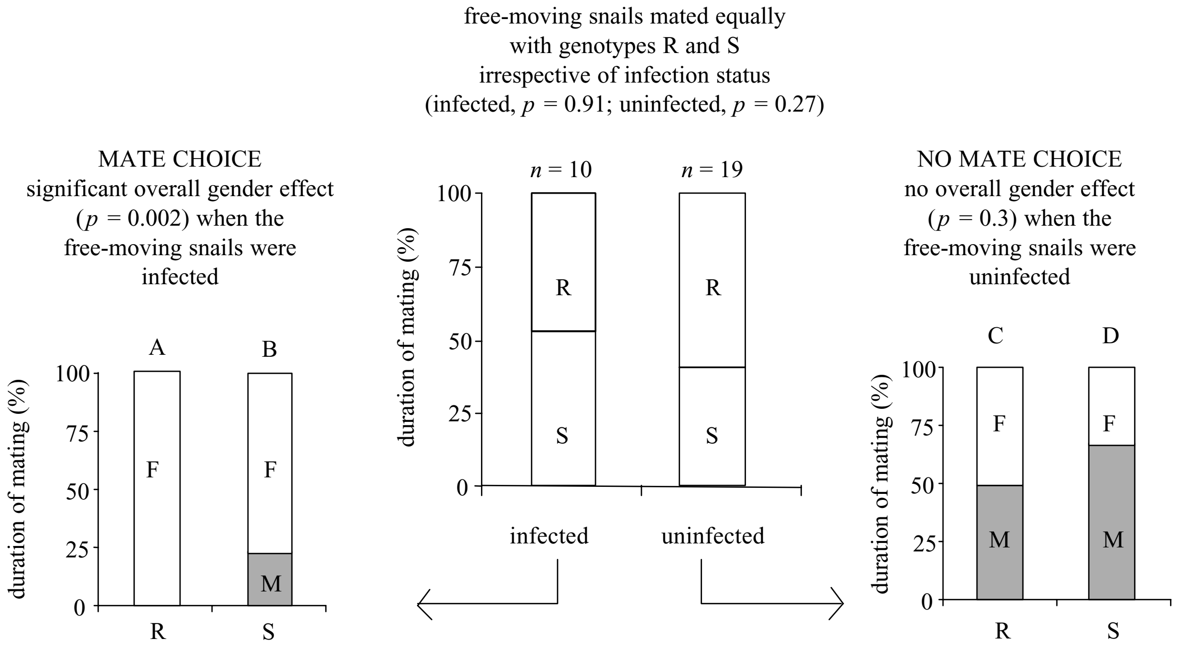

Figure 3. Mate choice on the basis of host resistance genotype and *S. mansoni* infection status. Duration of mating (expressed as the proportion of the total time) and gender adopted (M represents mating in the male gender and F represents mating in the female gender) by the free-moving *B. glabrata* snails of each infection status (uninfected or infected with *S. mansoni*) when mating with the tethered snails of each genotype (R, resistant-selected snails; S, susceptible-selected snails). Mating was recorded when the penis of one snail was visibly inserted into the genital pore of its partner. Free-moving snails showed a significant gender bias, mating more often as females with either genotype when infected than when uninfected (Wilcoxon–Mann–Whitney: A versus C: $S = 202$, $p = 0.02$; B versus D: $S = 214$, $p = 0.001$). Clear mate choice was shown by the tethered snails when faced with an infected individual ($S = 58$, $p = 0.002$), and this depended upon genotype: resistant snails actively refused to copulate in the female gender with an infected partner ($p = 0.02$), while susceptible snails continued to copulate in either gender ($p = 0.5$) (see table 1).

$p = 0.91$; uninfected: $S = 332$, $p = 0.27$; figure 3). Clear mate choice, expressed as gender bias, was, however, shown by the tethered snails when faced with an infected individual ($S = 58$, $p = 0.002$), and this depended upon genotype: resistant snails refused to copulate as female with an infected partner (figure 3*a*), while susceptible snails copulated in either gender (figure 3*b*). By contrast, no overall gender biases were shown when the free-moving snails were uninfected ($S = 326$, $p = 0.3$). These differences in gender biases can be understood in terms of:

(i) the gender adopted at the start of each mating bout (snails averaged 2.3 mating bouts per night, each lasting a mean of 45 min); and
(ii) the level of subsequent reciprocation (gender swapping) within the same mating bout (table 1).

Thus, the lack of overall gender bias among uninfected snails (figure 3*c*,*d*) was caused by the fact that they were equally likely to initiate a mating bout as male or female, and subsequently reciprocated in equal measure with either genotype (table 1*c*,*d*). By contrast the overall gender bias among infected snails was caused by their general willingness to act as a female during the first sexual encounter (all infected snails initiated mating bouts as females: 10/10, binomial test, $p = 0.001$), while the difference between genotypes was caused by the fact that infected snails were able to swap gender (i.e. mating as male through reciprocation) only when mating with the susceptible partners (table 1*b*), whereas none of the resistant snails allowed reciprocation when the free-moving snail was infected (figure 3*a*; table 1*a*). Indeed, as a result of the resistant snails' persistent efforts to mate as male ($F_{1,16} = 10.07$, $p = 0.006$ on 'penis probing' behaviour (resistant vs. non-resistant), whose duration was positively correlated with subsequent male mating success across all snails (Spearman's $r = 0.43$, $p = 0.004$)), the infected free-moving snails 'accepted' to act as females for twice as long as they would normally do (infected snails did not terminate the copulation bouts; only resistant snails terminated copulation through shell swinging or retraction).

There is no indication that the fact that all infected snails initiated the first mating bout as females was the result of an increased initial willingness to do so. Rather the data suggest that even the first mating encounters were the outcome of conflicts between mating partners who required an increased probing effort before the infected snails accepted to mate as female ($F_{1,25} = 3358$, $p = 0.008$). This was particularly pronounced with resistant snails, who had to probe for over twice as long (mean of 58 min per snail) to mate exclusively as males ($F_{1,25} = 3793$, $p = 0.005$).

4. DISCUSSION

The results show that mate choice during hermaphroditic sex is not under the control of one individual, even when one snail is able to move between partners. Rather, the outcome depended upon both mating partners, was modulated by the combination of potential and current

Table 1. Proportions (with associated p-values) of mating bouts that the free-moving snail: (i) 'initiated' as a female (where 'initiated' refers to the gender adopted at the start of the mating bout); and (ii) subsequently mated as a male (reciprocation), depending upon its infection status and the genotype of its partner.
(Mating was recorded when the snail's penis was visibly inserted into the other snail's genital pore. The table shows that the significant overall gender bias among infected snails (figure 3) is caused by the fact that they always initiated mating as a female with both (*a*) resistant and (*b*) susceptible partners. Infected snails mated as males only via subsequent gender swapping within the same mating bout (reciprocation) and only with susceptible snails. (*c*,*d*) By contrast, uninfected snails initiated mating bouts as either gender, and were able to reciprocate, with both genotypes; p-values refer to the significance of the deviation from an expected 50% (binomial tests).)

	infected free-moving snail mates:		uninfected free-moving snail mates:	
	(*a*) with resistant	(*b*) with susceptible	(*c*) with resistant	(*d*) with susceptible
(i) mating bouts initiated as female	100% $p = 0.02$	100% $p = 0.01$	50% $p = 0.5$	36% $p = 0.14$
(ii) subsequent gender swaps (reciprocation) within the same mating bout	0% $p = 0.02$	57% $p = 0.5$	54% $p = 0.5$	62% $p = 0.36$

infection statuses of the mating dyad, and was determined by the more subtle effects of gender biases rather than by absolute levels of mating activity.

Adaptive gender expression in simultaneous hermaphrodites should reflect the relative costs and benefits of each sexual function. As always, however, one must first distinguish host from parasite effect. It is unlikely that the parasite is manipulating the host's sexual role here, since mate choice was determined by the resistant snails, who were uninfected, rather than by the infected snails. The only apparent effect of infection status on the free-moving unselected snails was that infected snails always 'accepted' to start copulating as females, but this was equal across both resistant and susceptible partners. Moreover, such female bias among infected snails would be of no obvious benefit to the parasite, since schistosomes compete with the hosts' egg-production capacities for space and energetic requirements (Woolhouse 1989); the parasite would therefore feasibly benefit from biasing the mating of its infected host towards the male, rather than the female, gender. The gender biases observed here appear instead to reflect the continuum of cost–benefit trade-offs to the host under the various infection and genotype situations tested. It is generally assumed that the preferred role in simultaneous hermaphrodites is to mate as a male since sperm production, as in most sexual species, is believed to be less costly than egg production (Bateman 1948; Charnov 1979). When interests are identical and incompatible, gender cost differences within hermaphrodites are thus expected to lead to conflicts, with both snails vying to mate in the preferred male role (Bateman 1948; Charnov 1979; Wethington & Dillon 1996; Michiels 1998). However, game-theory models based on the Prisoner's Dilemma (Rapoport & Chammah 1965) and the Hermaphrodite's Dilemma (Leonard 1990) predict that the outcome should be one of cooperation (i.e. sperm trading via reciprocal gender swapping) rather than defection (i.e. mating in the preferred male role only; Leonard 1991; Michiels 1998). Our results confirm these general predictions when the free-moving snails are uninfected: reciprocation was common, with 74% of snails observed to switch gender at least once and up to three times during a mating bout. Furthermore, as predicted under these models, even within a context of general cooperation, the spectre of defection still loomed large (Nowak & Sigmund 1993): within any given mating bout, the probability of one or more reciprocations was consistently less than 1 (table 1). Uninfected snails, who initiated copulations as males and females in equal measure with both genotypes, were unaffected by this less-than-perfect level of reciprocation. But lack of reciprocation was responsible for the total female bias observed among infected snails mating with resistant partners—as all infected snails, having copulated first as females, relied completely on reciprocation to mate as males (table 1*a*,*b*).

Both causal and functional arguments are consistent with the observation that all infected snails always 'accepted' to start copulations as females. Causally, infected snails may be less capable of winning male–male conflicts (characterized here by simultaneous penis probing, resulting from both members of the dyad vying to mate in the male gender, and penis biting and shell swinging to prevent insertion) owing to the morbidity of schistosome infection (Woolhouse 1989; Webster & Woolhouse 1999). Susceptible snails may simply be unable to detect the infection status of their partners, suggesting that the mechanism by which snails recognize infected partners could be similar to that by which they resist parasitic infection. An alternative explanation is that susceptible snails do recognize the infection status of their partner, but that they gain more by choosing to reciprocate, since maximizing outcrossing through both genders would be favoured here as genotypic diversification among sexually reproduced progeny could help them to evade coevolving parasitism through a rare advantage (Bateson 1983; Hamilton *et al.* 1990; Read 1990; Jennions & Petrie 1997). By contrast, owing to the heritability of resistance and the high cost, in terms of increased mortality, of infection (Webster & Woolhouse 1998, 1999), resistant snails would benefit by discriminating against accepting sperm, and hence 'inferior' genes (i.e. infectable phenotype) from an infected partner. Such a strategy would avoid the disadvantages of 'outcrossing depression', owing to the immediate break-up through recombination of successful co-adapted resistance-gene complexes, through their extremely costly female gender, a trait exacerbated by the reduced egg viability of these resistant snails (Woolhouse 1989; Webster & Woolhouse 1999). In game-theory terms, resistant snails thus defected by refusing to reciprocate, outcrossing exclusively in the favoured male gender, and leaving open the option to self in the female role.

The results presented here may also have implications for the evolution of recognition systems. The mating patterns observed among resistant snails presented with an infected partner suggest the existence of both mate recognition and discrimination on the basis of the partner's infection status as well as a potentially differential sexual strategy, in terms of outcrossing or selfing, based upon the individual's own resistance genotype. How this is achieved can only be speculative at this stage but one plausible mechanism involves the recognition of chemical compounds in the snail's mucous or shell composition, which may be sampled during the mutual allo-grazing periods that preceded copulation. If confirmed, then the similarity to suggestions of odour-mediated mate choice for major histocompatibility complex (MHC) status in mammals is apparent (Wedekind *et al.* 1995; Penn & Potts 1998). This may therefore lend support to the theory that complex self–non-self recognition systems in vertebrates, such as that of the MHC, evolved from these conceptually simple, but apparently highly efficient, invertebrate recognition systems (Brown & Ecklund 1994).

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