Mate choice, frequency dependence, and the maintenance of resistance to parasitism in a simultaneous hermaphrodite

Joanne P. Webster1 and Charlotte M. Gower

Department of Infectious Disease Epidemiology, Imperial College Faculty of Medicine, Norfolk Place, London W2 1PG, UK

Synopsis Biomphalaria glabrata are simultaneous hermaphroditic freshwater snails that act as intermediate hosts for the macroparasitic trematode Schistosoma mansoni, a causative agent of schistosomiasis. Heritability and strain-specificity of both snail resistance and susceptibility to schistosome infection have been demonstrated, genetic variability for which is maintained, in part, through trade-offs between high fitness costs associated with infection and those associated with resistance. However, despite such a high cost of resistance and a low prevalence of infection in natural snail populations, genes for resistance are maintained within snail populations over successive generations, including in the complete absence of parasite pressure in laboratory populations. This may be indicative of alternative benefits of resistance genes, in addition to parasite defense, such as differential mating success between genotypes. Here we examined the mate and gender choice of snails across a multi-factorial range of potential partner combinations. These included host-resistance or susceptibility genotype, host genotype frequency within the population, current parasite infection status, and parasite genotype. We demonstrate recognition and discrimination by host snails depending on host and/or parasite genotype for each of these factors. In particular, our results suggest that a rare mating advantage to resistant genotypes may be a potential explanation for the maintenance of highly costly resistance genes within intermediate host populations under conditions of low or zero parasite pressure.

Introduction Biomphalaria glabrata are simultaneous hermaphroditic freshwater snails that act as intermediate hosts for the macroparasitic trematode Schistosoma mansoni, a causative agent of schistosomiasis. Schistosomiasis ranks second only to malaria in terms of parasite-associated human morbidity and mortality, with over 200 million people currently infected. Transmission between hosts occurs via free-swimming larval stages, miracidia (infective to the mollusc) and cercariae (infective to the mammal). Prevalence and transmission of schistosome infections in natural populations is highly variable across space and time, explicable in part by ecological and genetic aspects of interactions between schistosomes and their intermediate hosts (Wilkins 1987; Webster and Davies 2001). Although the precise molecular underpinnings have not yet been identified, snail resistance and susceptibility have been demonstrated to have a heritable, strain-specific basis (Webster and Woolhouse 1998; Webster 2001). Variability is maintained within natural snail metapopulations, in part, through trade-offs between high fitness costs resulting from parasitism and those associated with resistance: susceptible snails suffer from significantly increased mortality when infected, whereas resistant individuals suffer significantly reduced egg viability (Woolhouse 1989; Cooper and others 1994; Cousin and others 1995; Webster and Woolhouse 1999). However, despite such a high cost of resistance and a low prevalence of infection in natural snail populations (Webster and others 2001b), genes for resistance appear to be maintained within snail populations over successive generations, including in the complete absence of parasite pressure within laboratory populations (Webster and Woolhouse 1998; Webster 2001). Such maintenance of resistance polymorphism suggests alternative benefits of resistance genes in addition to those of parasite defense, a possibility of which could be differential mating success between genotypes.

Hamilton and Zuck’s landmark article (Hamilton and Zuck 1982) alerted many biologists to the possibility that parasites were of fundamental importance in the evolution and maintenance of active mate choice...
Such potential for mixing of host and parasite genotypes within natural populations may be predicted to influence potential host mate choice and sexual strategies particularly where, for instance, the virulence of parasite infections vary (Webster and others 2004) and/or in relation to the frequency of specific genotypes within the population.

Frequency-dependent sexual selection has received considerable attention in the scientific literature and in textbooks and empirical evidence from plants and a few animal systems, notably fruit flies, has been reported (Partridge and Hill 1984; Ross 1984; Partridge 1988). It is a mechanism which can contribute to the maintenance of genetic variability in a population when the selective values are inversely related to the genotypic frequencies. Thus, when a rare genotype has a higher fitness than the common type, selection may lead to a protected polymorphism. Initially the rare genotype will increase in frequency, if there are no other selective forces operating against it, but as soon as the rare type becomes more common, its advantage is reduced which may lead to stable oscillation. A major advantage of frequency-dependent sexual selection is that it can provide a means for maintaining genetic variability within populations without incurring a large genetic load.

Negative frequency-dependence is classically measured as a rare-male effect (RME) and/or the cross product ratio (CPR) (Partridge and Hill 1984; Partridge 1988), such that rare males have increased mating success than would be expected by chance alone. Whilst, as may be termed here, a “rare-snail effect” (RSE) may be more directly applicable to a simultaneous hermaphroditic snail species than a RME, such frequency-dependent advantage for rare genotypes may well be predicted. In models of dynamic or Red Queen coevolution of host-resistance and parasite infectivity (Hamilton 1980; Morand and others 1996), parasite genotypes track common host genotypes, promoting a frequency-dependent advantage to rare host genotypes, as they escape the deleterious consequences of parasitic infection. As the rare-snail genotype and their progeny would have a lower risk of infection, it may be predicted that there should be a selective benefit to any snail able to preferentially mate with such a resistant partner. This should be the case whether the rare snail is either passively resistant as a virtue of being rare (that is not yet recognizable by the tracking parasite) or actively resistant (that is able to actively inhibit establishment or replication of specific parasite genotypes, but rare due to the inherent costs of such resistance). Indeed, there does appear to be evidence of Red-Queen evolution and coevolution in action.
within the snail-schistosome system (Woolhouse and Webster 2000; Webster and Davies 2001; Webster and others 2004), and thus frequency-dependent mate choice for rare genotypes may well be predicted as a plausible mechanism to explain the maintenance of resistance polymorphism in host populations independent of current parasite pressure.

Here we performed a series of laboratory experiments examining the differential roles and interplay of host and parasite phenotype and genotype on snail mate choice. In particular, across a multi-factorial range of potential partner combinations, we aimed to elucidate whether host genotype frequency within the population had any differential impact upon mating success (RSE), and whether this was influenced by either the host-resistance or susceptibility genotype and/or that of the parasite infection and parasite genotype status.

**Materials and methods**

**Host–parasite maintenance**

Laboratory strains of *B. glabrata* snails and *S. mansoni* parasites were used. Snail and parasite lines had been maintained in the laboratory for many (each >20) generations, but had been demonstrated to be genetically polymorphic (Webster and Woolhouse 1998b; Webster 2001; Webster and others 2004). Snails were maintained in the laboratory at 23–26°C, and subject to a light (full UV spectrum; SAD lightboxes Co. Ltd) regime of 12 h light (between 8 AM and 8 PM) and 12 h darkness. Snails were kept in plastic tanks containing natural mineral water (Ice Valley®). The tanks were changed weekly, and snails were fed *ad libitum* on fresh lettuce and fish food (Tetra). All snail tanks contained Styrofoam sheets, on which the snails would preferentially deposit their eggs. Snails for experimental groups were exposed individually to 5 *S. mansoni* miracidia, in approximately 6 ml of dechlorinated water for 2 h. Control snails were treated identically but without parasite exposure. In order to determine the infection status of snails, snails were placed in darkness for 48 h from 4 weeks post *S. mansoni* exposure (or unexposed controls), and then placed in individual vials with 20 ml of distilled water and placed them in light provided by a 60 W lamp for 2 h.

In the following experiments, snails described as resistant refer to those snails exposed to *S. mansoni* miracidia but which did not subsequently shed cercariae at any stage (and did not contain sporocysts on squashing at the end of study), indicative of a lack of parasite establishment. “Susceptible” snails are referred to here as those exposed snails which did subsequently shed cercariae (and did contain sporocysts), indicative of successful parasite establishment. Such groups are thus distinct from the resistant-selected but unexposed, and susceptible-selected but unexposed, snails of the previous studies (Webster 2001, 2002; Webster and others 2003). Control snails were those unexposed and uninfected.

**Experiment 1: impact of host frequency and host-resistance genotype**

*B. glabrata* (Bg-PR: an albino strain originating from Puerto Rico) were removed from their stock lines 4 weeks prior to each trial and kept in isolation to avoid potential bias in allo- and auto-sperm storage (Paraense 1956). Snails were then placed in plastic rectangular mating arenas (4 × 6 cm) filled with water, but without food. Each arena contained 5 free size-matched and sexually mature snails: 4 common snails to 1 rare snail, with each possible combination of resistant, susceptible, and unselected controls (Fig. 1). For each combination 5 replicates were performed, thereby providing 30 trials in total. Each snail was individually identified using a (randomly chosen) color-coded number (previously demonstrated not to impact host behavior). In all trials, all snails within each quintuplet were previously unknown to each other.
other (that is all had been maintained in separate stock-line tanks prior to these experimental trials).

Observations were recorded directly by continuous sampling within 5-min intervals over 125 min per trial coding for the following measures: penis insertion into its partner’s genital pore (copulating in the male gender); being inserted by a partner (copulating in the female gender); self-insertions (penis inserted into own genital pore); penis probing; penis hanging; grazing another snail (where the snail appears to be grazing the outer surface of its partners shell, an act which often precedes copulation); being grazed; touching/in contact with another snail (but without mating or grazing); being touched; isolated (>1 cm in diameter from any other snail); avoided contact/broke away (retracting or biting partner’s penis).

Total mating activity was measured as the number of mating events in the arena during the trial. The CPR was calculated separately in the male and female gender according to the equation [adapted from (Partridge 1988)]:

\[
\frac{(\text{No. of genotype A snails mating}) \times (\text{no. of genotype B snails present})}{(\text{No. of A snails present}) \times (\text{no. of B snails present})}.
\]

The number of penis-probing events, avoidance behaviors, self-insertions, and copulations in the male and female gender were calculated for common and rare snails. Non-continuous involvements in the same behavior with the same partner were considered separate events. An estimate of the time spent grazing, being grazed, touching, being touched, penis probing, mating as male, mating as female, isolated, and penis hanging was taken as the number of 5-min time intervals during which the behavior was recorded. Thus, for example, if a snail grazed another for 3 time intervals (up to 15 min in total duration), that snail was recorded as having a grazing duration of 3. Behavior duration data were estimated as the proportion of total time available during the trial for rare and common snails and thus was directly comparable between rare and common genotypes, controlling for the fact that there were 4 common but only 1 rare snail.

Summary statistics were calculated for each replicate trial. Analysis of variance was used to compare the total mating activity and CPRs using genotype of rare snail (resistant, susceptible, or control), genotype of common snail (resistant, susceptible, or control) and their interactions as dependent variables. The presence of negative frequency-dependent mate choice (RSE or rare advantage) was investigated for each compatibility genotype in turn, by comparing the set of CPR in trials when the genotype of interest was the rare snail with those when the genotype of interest was the common snail. Mann-Whitney U-tests were used to determine if the CPR was higher when the genotype was rare rather than common, and thus if rare snails mated more frequently than would be expected by chance alone. Analysis of variance was used to compare the frequency and duration of behaviors by rare and common snails observed during the 30 replicate trials with rarity (rare or common), genotype of rare snail (resistant, susceptible, or control), genotype of common snail (resistant, susceptible, or control) and their interactions as dependent variables. Proportion data were arc-sin transformed prior to analysis.

**Experiment 2: impact of host frequency and parasite genotype**

This experiment directly investigated active mate choice in relation to host frequency and infection-status interactions between susceptible host snails infected with either a high (HIGH) or low (LOW) infection intensity parasite genotype, or uninfected control snails. We aimed to determine if the genotype of the parasite infecting susceptible snails affected the mate choice, and/or if different types of infection provide varying pressure for active mate choice in this system. Inbred parasite strains were developed from a parental laboratory strain of *S. mansoni* of mixed geographical origin, by the cross-breeding of single clones of female schistosomes to single male schistosome clones, as has been described in detail elsewhere (Davies and others 2001). We selected 2 strains with repeatable differences in their average infection intensity phenotype in the snail host (measured as the number of cercariae produced) for use in this study. Infection intensity in the HIGH intensity strain was approximately 4-fold higher than in the LOW intensity strain [mean weekly number of cercariae produced per snail in a sample of 30 snails exposed to 6 miracidia per snail of each parasite strain was 608.9 ± 106.3 for the HIGH intensity strain and 148.2 ± 96.9 for the LOW intensity strain and this was a significant difference (F \(_{1,50} = 13.95, P<0.001\)).]

Previous research on *B. glabrata–S. mansoni* interactions has determined that there is a relationship between infection intensity and the virulence (measured as a reduction in host longevity) although, perhaps counter-intuitively, this is a negative relationship such that HIGH intensity infections are less virulent to the snail host than LOW intensity infections. This is possibly due to high costs inherent within the immune defense response of snails, where a lack of mounting such a response results in reduced virulence (Davies and others 2001; Gower and Webster 2004).
As for experiment 1, each arena contained 5 free size-matched sexually mature snails: 4 common snails to 1 rare snail, with each possible combination of snails infected with HIGH or LOW infection intensity parasite genotypes, or unexposed and uninfected CONTROL snails. Identical statistical analyses were conducted as for experiment 1, but using the genotype of the infecting parasite (HIGH, LOW, or CONTROL) in place of the compatibility genotype of the snail.

**Results**

**Experiment 1: impact of host frequency and host-resistance genotype**

**Mating incidence**

The CPRs, demonstrating individual mating success, are shown in Table 1. For copulations in the male gender, there was no overall difference in the CPR depending on rare \( F_{2,48} = 1.48, P = 0.25 \) or common genotype \( F_{2,48} = 0.38, P = 0.69 \), or their interaction \( F_{1,48} = 0.61, P = 0.44 \). However, the key question in determining the presence of negative frequency-dependent mate choice (rare advantage) is whether the ratio is higher for a particular genotype when it is rare rather than common. For resistant snails, there was marginally significant evidence of rare advantage as the CPR was higher when resistant snails were rare than when common \( W = 105.5, P = 0.07 \). In contrast, there was a trend toward a rare disadvantage for susceptible snails \( W = 99.5, P = 0.07 \), such that they mated less frequently than would be expected when they were rare. There was no effect of frequency in the population on the mating success of control snails \( W = 108.0, P = 0.44 \). In the female gender, there was, however, no overall difference in CPR depending on host genotype, and neither was there evidence of rare advantage for any of the genotypes (resistant snails \( W = 91.5, P = 0.47 \); susceptible snails \( W = 114.0, P = 0.47 \); control snails \( W = 107.0, P = 0.41 \)).

The number of copulations in the female gender was significantly higher for common than rare snails (as would be expected given they are 4 times as common) \( F_{2,48} = 17.5, P < 0.001 \) but this was not influenced by the compatibility genotype of the common \( F_{2,48} = 1.39; P = 0.26 \) or rare snails \( F_{2,48} = 2.68, P = 0.08 \). Almost identical results were observed for the number of times mating in the male gender. Self-insertions were a very rare event, only being observed once in the study (when the rare, self-inserting snail was susceptible and the common snail was resistant).

Mean time to initiation of mating as male (that is time spent touching, grazing, probing before penis insertion) was significantly longer in rare snails \( F_{1,48} = 24.9, P < 0.001 \) but was not affected by the common genotype \( F_{2,48} = 0.76, P = 0.48 \). However, the rare genotype was important, being significantly longer for control snails \( F_{2,48} = 17.7, P < 0.001 \). There was also a significant interaction term (rare-common * raregenotype \( F_{2,48} = 12.9, P < 0.001 \), such that this effect was only observed when control snails were rare.

**Mating duration—proportion of total time spent copulating**

In the male gender, common snails mated significantly more than rare snails \( F_{1,48} = 7.96, P = 0.007 \), despite the fact that their commonality has been accounted for in the analysis. The time spent mating as a male was not significantly affected by the genotype of rare \( F_{2,48} = 0.63, P = 0.54 \) or common \( F_{2,48} = 0.27, P = 0.76 \) snails. However, there was a significant interaction term such that the increased success of the common snails over the rare snails did not occur when the rare snail was resistant \( F_{1,48} = 4.22, P = 0.05 \), indicative of a rare advantage to resistant snails (Fig. 2a).

There was no significant difference, however, between the total time spent mating in the female gender according to rarity status \( F_{1,48} = 0.271, P = 0.61 \), compatibility genotype of rare snail \( F_{2,48} = 1.18, P = 0.31 \), common snail \( F_{2,48} = 0.335, P = 0.72 \), or their interactions (Fig. 2b).

**Interaction behavior**

Whilst there was no effect of rarity \( F_{1,48} = 0.24, P = 0.63 \), or rare genotype \( F_{2,48} = 1.02, P = 0.37 \), on the proportion of total time spent grazing, the proportion of total time spent grazing was

---

**Table 1** Mean (± standard error) CPRs for combinations of resistant, susceptible, and control snails in mating trials containing 4 common snails and 1 rare genotype snail

<table>
<thead>
<tr>
<th>CPR male gender</th>
<th>Common snails</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
<td>Control</td>
</tr>
<tr>
<td>Resistant</td>
<td>n.a.</td>
<td>2.00 ± 0.82</td>
<td>1.50 ± 0.86</td>
</tr>
<tr>
<td>Susceptible</td>
<td>0.40 ± 0.40</td>
<td>n.a.</td>
<td>0.22 ± 0.22</td>
</tr>
<tr>
<td>Control</td>
<td>0.80 ± 0.80</td>
<td>0.80 ± 0.80</td>
<td>n.a.</td>
</tr>
<tr>
<td>CPR female gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>n.a.</td>
<td>2.50 ± 0.96</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Susceptible</td>
<td>1.20 ± 0.80</td>
<td>n.a.</td>
<td>1.56 ± 0.80</td>
</tr>
<tr>
<td>Control</td>
<td>0.80 ± 0.80</td>
<td>0.80 ± 0.80</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

CPRs were conducted separately for copulations in the male and female gender. Each value represents the mean of 5 independent replicate trials.

n.a.—not applicable to all snails of same genotype.
significantly affected by common genotype, where resistant snails spent significantly more time grazing than either susceptible or control snails ($F_{2,48} = 5.45, P = 0.007$).

In terms of being grazed by another snail, common snails were also grazed more often than rare snails ($F_{1,48} = 5.36, P = 0.03$) and the genotype of the common snails was significant, where resistant snails were grazed more often than either control or susceptible snails ($F_{2,48} = 3.92, P = 0.03$). Again, the genotype of the rare snail was not important ($F_{2,48} = 0.36, P = 0.70$).

There was no effect of rarity ($F_{1,48} = 2.04, P = 0.16$) or common genotype ($F_{2,48} = 0.59, P = 0.56$) on the proportion of total time spent touching another snail. However, this was significantly affected by

![Fig. 2 Proportion of total time spent mating as (a) male and (b) female for common and rare snails in various host compatibility genotype combinations, where either resistant (red), susceptible (yellow), or control (green).](image-url)
genotype, where resistant snails spent more time touching than either control or susceptible snails \((F_{2,48} = 5.55, P = 0.007)\). Similarly, resistant snails spent more time being touched than control or susceptible snails \((F_{2,48} = 5.18, P = 0.009)\).

Rare snails spent significantly more time isolated than common snails \((F_{1,48} = 24.67, P < 0.001)\), indeed common snails were never classified as isolated during the experiment. The genotype of the rare snail made no difference as to the duration of time spent isolated \((F_{2,48} = 0.72, P = 0.49)\). However, the common genotype significantly influenced the amount of time spent isolated by the rare snail \((F_{2,48} = 5.31, P = 0.008)\) being significantly reduced when the common snails were control genotype rather than resistant or susceptible.

Active avoidance behaviors (retraction, penis biting, shell swinging) were relatively uncommon (mean number of events per mating trial = 0.25) but all those observed were directed toward common snails. This was not significantly affected by the compatibility genotype of the rare \((F_{2,48} = 0.15, P = 0.86)\) or common snails \((F_{2,48} = 0.58, P = 0.56)\).

Thus in summary, common snails mated more often as males than rare snails, even accounting for their commonality. However, when the rare snail was resistant, this effect was diminished, and indeed resistant snails showed evidence of negative frequency-dependent mate choice, being relatively more successful when rare than when common. Rare snails spent more time isolated and took longer from initiation of contact to achievement of mating. No differences were observed for mating in the female gender. In general, resistant snails were more involved in touching and grazing other snails than control and susceptible genotypes, but this did not translate to an overall increase in mating success of resistant snails. Rather resistant snails did not suffer the disadvantages of rarity experienced by common and susceptible snails, and instead had a rare advantage.

**Experiment 2: impact of host frequency and parasite genotype**

**Mating incidence**

The CPRs are shown in Table 2. For the mating success in the male gender, there was no overall difference in the CPR depending on rare \((F_{2,24} = 0.59, P = 0.56)\) or common genotype \((F_{2, 24} = 0.75, P = 0.48)\) or their interaction \((F_{1,24} = 0.02, P = 0.88)\). However, LOW-infected snails \((W = 135, P = 0.01)\) had a rare advantage and mated significantly more frequently when rare than would be expected by chance. In contrast, there was no evidence that HIGH-infected \((W = 90.0, P = 0.14)\), or uninfected control snails \((W = 92.0, P = 0.17)\) mated more frequently by virtue of their rarity. In the female gender, there was no overall difference in CPR depending on host genotype, nor any evidence of frequency-dependent mate choice in LOW-infected snails \((W = 106.0, P = 0.48)\) respectively. However, the HIGH-infected snails showed a non-significant trend toward rare advantage \((W = 122.5, P = 0.07)\) and the uninfected control snails toward rare disadvantage in terms of mating incidence \((W = 87.0, P = 0.07)\).

The total number of copulations during the trial in the male gender was significantly higher for common \((F_{1,48} = 19.4, P<0.001)\) than rare snails (as would be expected given that there were 4 common snails but only 1 rare snail) but was not influenced by the infection status of the common \((F_{2,48} = 0.34, P = 0.71)\) or rare snail \((F_{2,48} = 1.72, P = 0.19)\). Similarly, although the number of matings in the female gender was significantly higher for common than rare snails \((F_{1,48} = 19.9, P<0.001)\) it was not influenced by the common genotype \((F_{2,48} = 0.44, P = 0.65)\) or rare genotype \((F_{2,48} = 1.76, P = 0.18)\). Self-insertions were only observed during 1 trial, where a common snail infected with the LOW parasite strain repeatedly self-inserted.

**Mating duration—proportion of total time spent copulating**

In the male gender, mating duration was highest when the uninfected CONTROL line was the common snail \((F_{2,48} = 3.73, P = 0.03)\), but unaffected by rarity \((F_{1,48} = 0.04, P = 0.84)\) (Fig. 3a). In the female gender, mating duration was again significantly the highest amongst the CONTROL line when common \((F_{2,48} = 3.88, P = 0.03)\), but was also significantly

---

**Table 2.** Mean (± standard error) CPRs for combinations of HIGH-infected, LOW-infected and uninfected CONTROL snails in mating trials containing 4 common snails and 1 rare genotype snail.

<table>
<thead>
<tr>
<th>Rare snail</th>
<th>Common snails</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIGH</td>
</tr>
<tr>
<td>CPR male</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>n.a.</td>
</tr>
<tr>
<td>LOW</td>
<td>1.67 ± 0.67</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1.07 ± 0.78</td>
</tr>
<tr>
<td>CPR female</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>n.a.</td>
</tr>
<tr>
<td>LOW</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.40 ± 0.40</td>
</tr>
</tbody>
</table>

CPRs were conducted separately for copulations in the male and female gender. Each value represents the mean of 5 independent replicate trials. n.a.—not applicable to all snails of same genotype.
increased amongst HIGH-infected snails when rare $(F_{2,48} = 3.06, P = 0.05)$ (Fig. 3b).

Interaction behavior
Common snails showed a marginally significant trend toward increased grazing $(F_{1,48} = 3.59, P = 0.06)$, but neither the infection status of the rare snail $(F_{2,48} = 0.44, P = 0.65)$ or common $(F_{2,48} = 1.13, P = 0.33)$ had an overall effect on the proportion of total time spent grazing. However, there was a significant interaction term and the amount of grazing depending on the combination of rare and common
Snail sexual strategies

snails ($F_{1,48} = 7.25, P = 0.01$). In terms of being grazed by other snails, HIGH-infected rare snails were more likely to be grazed ($F_{2,48} = 4.07, P = 0.02$) than LOW-infected or CONTROL snails but the interaction of rare and common snail was again important ($F_{1,48} = 8.39, P = 0.006$). There were no significant differences between replicates in the proportion of total time spent touching other snails.

Active avoidance behaviors (retraction, penis biting, shell swinging) were almost 5 times more common than in previous experiment 1 (mean number of events per mating trial = 1.28). There was a non-significant trend of increased avoidance directed at common snails when the rare snail was infected with HIGH intensity parasites ($F_{2,48} = 2.53, P = 0.09$) with the lowest number of avoidance being directed toward the uninfected CONTROL line. This was true of both rare and common snails ($F_{1,48} = 0.003, P = 0.96$) and was not influenced by the infection status of the common genotype ($F_{1,48} = 0.21, P = 0.81$).

Thus in summary, in common with experiment 1, rare snails spent more time isolated than common snails, although there was insufficient evidence of a reduction in mating success attributable to this. Similarly, we also observed evidence of frequency-dependent mate choice, where LOW-infected snails had a rare advantage in the male gender, whereas HIGH-infected snails had a rare advantage in the female gender. This was also apparent as an increase in the duration of mating as a female when rare snails where infected with the HIGH intensity parasite genotype. Uninfected CONTROL snails were more successful, in terms of both mating incidence and duration, when common.

Discussion

Our results here show evidence of recognition and discrimination for mate choice and sexual strategy by *B. glabrata* snails on the basis of host-resistance genotype, frequency within the population, infection status, and also the genotype of the infection status. In particular, our results suggest that a rare mating advantage to resistant genotypes may be a potential explanation for maintenance of highly costly resistance genes with host populations, even under conditions of low or zero parasite pressure.

It has been stated that frequency-dependent mate choice would be a curious finding from an evolutionary point of view, as if male morphs gain a mating advantage when rare, yet stay rare, then some effect reducing fitness must balance the mating advantage (Partridge and Hill 1984). This potential complexity may, however, be counteracted within the snail-trematode system as rare snails (as distinct from males alone) have previously been demonstrated to not stay rare, but become common in subsequent generations (Dybala and Lively 1998), and there are clear fitness trade-offs that can balance mating advantages, in terms of costs of resistance and costs of susceptibility. This may indeed highlight an advantage of studying potential frequency-dependent mate choice in hermaphrodite snails (to the authors’ knowledge, here for the first time for any hermaphroditic animal), as we can observe gender effects within strain and species and we know something of the relative costs between genders.

Across both our experiments, there was no evidence of increased mating success of rare snails *per se*, such as might be expected if rarity was being used as a surrogate for potential passive resistance (in contrast to active resistance, as defined earlier). In fact, the opposite was true and in general the common snails were advantaged in terms of their interaction with other snails, and in terms of direct mating success. Nevertheless, a form of frequency-dependent mate choice was observed, and was influenced by both host genotype and the genotype of the infecting parasite strain.

In the first experiment, common snails mated more often as males than rare snails, even accounting for their commonality. However, when the rare snail was resistant, this effect was diminished, and indeed resistant snails showed evidence of negative frequency-dependent mate choice, being relatively more successful when rare than when common. Resistant snails were also more involved in touching and grazing other snails than control and susceptible genotypes. Thus whilst resistant snails did not benefit from an overall increase in mating success *per se*, rather resistant snails did not suffer the disadvantages of rarity experienced by common and susceptible snails, and instead had a rare advantage. This could, therefore, be a mechanism for the maintenance of resistance genes in populations.
In the second experiment, it could have perhaps been predicted that no mate choice would occur, as in both experiment 1 and the previously published studies (Webster 2002; Webster and others 2003), it appears that only resistant genotype snails recognize and discriminate between potential partners, and in this study it was only the infecting parasite genotype that differed with host frequency. In contrast, there was a clear effect of infection status on mating success with uninfected control snails experiencing greater mating when common. Whilst it may be expected that the infected snails may have favored outcrossing in order to maximize recombination for their progeny, as mate choice is the consequence of both partners, our results appear to instead reflect an outcome of the combination of cooperation and conflict, where uninfected control snails were actually those refusing to copulate with an infected partner. Infected snails were, however, relatively more successful when rare, and the genotype of the infection influenced the gender of the rare advantage.

Gender also played an important role in the populations observed here across both studies. In the first experiment, whilst overall common snails mated more often as males than rare snails, such an increased success of the common snails over the rare snails did not occur when the rare snail was resistant. Adaptive gender expression in simultaneous hermaphrodites should reflect the relative costs and benefits of each sexual function, and the gender biases observed here appear to reflect the continuum of cost-benefit trade-offs to the host under the various infection/genotypes situations tested. It is generally assumed that the preferred role in simultaneous hermaphrodites is to mate as a male because 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 and Dillon 1996; Michiels 1998). Thus, as the preferred role indeed appears to be male in _B. glabrata_ (Webster and others 2003; Webster 2002), common snails have the advantage (in terms of a variety of equal potential partners), whilst the susceptible or control rare snails, for both of which previous molecular analyses have found 100% outcrossing if given the opportunity, may accept to copulate as female rather than forgo mating, because maximizing outcrossing would be favored here as genotypic diversification amongst sexually reproduced progeny could help them evade coevolving parasitism (Bateson 1983; Hamilton and others 1990; Read 1990; Jennions and Petrie 1997). In contrast, the resistant genotypes here appear to still be favored even if rare (Table 1), and thus continue to have the choice/opportunity to mate in the preferred male gender. Indeed our results here are also consistent with the previous molecular and behavioral studies that show resistant genotypes actively refuse to outcross in the female gender with a non-resistant partner and with subsequently partly self-fertilize instead (Webster 2001; Webster and others 2003). Indeed, the resistant snails’ avoidance of outcrossing in the female gender could also explain the lack of a RSE for resistant snails in the female gender observed here (Table 1). In this case, when rare, as a resistant genotype only has the opportunity to outcross with a non-resistant genotype, given the choice they would rather self (or forgo mating) than outcross in their costly female gender, exacerbated here by the costs of resistance in terms of decreased egg fertility (Webster and Woolhouse, 1999) (Fig. 2b), whilst continuing to outcross at the same frequency within their less costly male gender (Fig. 2a).

More difficult to explain, however, is why in experiment 2 snails infected with the HIGH-parasite genotype (low virulence) showed an increase in female mating while the LOW-infected (high virulence) had rare advantage in male gender. It could perhaps be speculated that infection with the LOW intensity parasite genotype, as the more virulent parasite, may have provoked a stronger immune response in those snails, and thus might be recognized by other snails as more similar to the resistant strains (for example, if they used some presence of immune molecules in the slime as the mechanism of discriminating and choosing). In contrast, the snails infected with the HIGH infection intensity genotype (LOW virulence) may appear obviously highly susceptible to infection, both to themselves and to potential partners, and may thus accept to mate more as females rather than forgo mating. In this case, such infected snails should want to maximize outcrossing and subsequent recombination, in contrast to the preferred part-selfing of the resistant snails of experiment 1. Alternatively, the rare advantage observed by the infected snails within experiment 2, irrespective of gender, may simply reflect the comparable reduced mating activity of these infected snails whilst common relative to their uninfected counterparts (Table 2; Fig. 3a and b).

**Conclusions**

Differential mate choice for, and by, resistance genotypes in a multi-host situation might promote further benefits explaining the maintenance of resistance
genes in populations, particularly those under low or zero parasite pressure. Although rarity per se appears to be disadvantageous to mating success, complex frequency-dependent interactions are occurring, where active mate choice appears to be based upon a subtle interplay between host rarity, gender, resistance genotype, and infecting parasite genotype. How this is achieved can only be speculative at this stage but a plausible mechanism involves the recognition of chemical compounds in the snail’s mucus or shell composition which may be sampled during the mutual grazing periods that preceded copulation. If confirmed, then the similarity to suggestions of odor-mediated mate choice for major histocompatibility complex status in mammals is apparent (Wedekind and others 1995; Penn and Potts 1998). Thus, whilst Charles Darwin wrote in his Descent of Man, “with animals belonging to the lower classes, the two sexes are not rarely united in the same individual, and therefore secondary sexual characters cannot be developed... Moreover it is almost certain that these animals have too imperfect senses and much too low mental powers to appreciate each other’s beauty or other attractions” (Darwin 1871), the hermaphroditic snail recognition system in terms of mate choice and sexual strategy, despite perhaps being conceptually simple, actually appears highly efficient and of great importance to the variability and success of these organisms.

Acknowledgments
We would like to thank Janet Leonard and the SICB for the invitation to present this work, Curt Lively for valuable discussion and input into the study, and the Royal Society and the Wellcome Trust for funding.

References