



HAL
open science

Effects of laboratory culture on compatibility between snails and schistosomes

A. Théron, C. Coustau, Anne Rognon, S. Gourbière, M.S. Blouin

► **To cite this version:**

A. Théron, C. Coustau, Anne Rognon, S. Gourbière, M.S. Blouin. Effects of laboratory culture on compatibility between snails and schistosomes. *Parasitology*, 2008, 135 (10), pp.1179-1188. 10.1017/S0031182008004745 . halsde-00330477

HAL Id: halsde-00330477

<https://hal.science/halsde-00330477>

Submitted on 14 Oct 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

2
3
4 **Effects of laboratory culture on compatibility between snails and schistosomes**

5
6
7
8 A. THERON¹, C. COUSTAU², A. ROGNON¹, S. GOURBIÈRE³, M. S. BLOUIN^{1,4*}

9
10
11 ¹*UMR 5244 CNRS-EPHE-UPVD, Biologie et Ecologie Tropicale et Méditerranéenne,*
12 *Université Via Domitia. 52 Av. Paul Alduy. 66860 Perpignan Cedex, France.*

13
14 ²*U547 Inserm, Institut Pasteur de Lille, 1 Rue du Prof. Calmette, BP 245. 59019 Lille Cedex,*
15 *France.*

16
17 ³*EA 3680, Mathématiques Et Physique pour les Systèmes (MEPS), Université de Perpignan*
18 *Via Domitia, 52 Av. Paul Alduy. 66860 Perpignan Cedex, France.*

19
20 ⁴*Dept. Zoology, Oregon State University, Corvallis, OR, 97331 USA*

21
22
23 *Corresponding author: Tel: 541-737-2362; Fax : 541-737-0501; email:
24 blouinm@science.oregonstate.edu

25
26 Running title: *schistosome-snail compatibility*

28 SUMMARY

29 The genetic control of compatibility between laboratory strains of schistosomes and their snail
30 hosts has been studied intensively since the 1970s. These studies show (1) a bewildering
31 array of genotype-by-genotype interactions – compatibility between one pair of strains rarely
32 predicts compatibility with other strains, and (2) evidence for a variety of (sometimes
33 conflicting) genetic mechanisms. Why do we observe such variable and conflicting results?
34 One possibility is that it is partly an artifact of the use of laboratory strains that have been in
35 culture for many years and are often inbred. Here we show that results of compatibility trials
36 between snails and schistosomes - all derived from the same natural population – depend very
37 much on whether one uses laboratory-cultured or field-collected individuals. Explanations
38 include environmental effects of the lab on either host or parasite, and genetic changes in
39 either host or parasite during laboratory culture. One intriguing possibility is that genetic
40 bottlenecks during laboratory culture cause the random fixation of alleles at highly
41 polymorphic loci that control the matched/mismatched status of hosts and parasites. We show
42 that a simple model of phenotype matching could produce dose response curves that look very
43 similar to empirical observations. Such a model would explain much of the genotype-by-
44 genotype interaction in compatibility observed among strains.

45

46

47 *Keywords: Schistosoma mansoni, Biomphalaria glabrata, resistance, susceptibility,*
48 *compatibility, parasite, host*

49 INTRODUCTION

50

51 Compatibility in host-parasite systems can be defined as the state in which a parasite can
52 establish infection and complete its development in a host (Basch, 1975). Thus, compatibility
53 is a joint trait of the parasite and host that probably depends on the genotypes of parasite and
54 host at many different loci. Consequently, there is ample opportunity for complex genotype-
55 by-genotype interactions: the phenotype of the host (susceptible/resistant) depends on the
56 genotype of the parasite, and the phenotype of the parasite (infective/noninfective) depends on
57 the genotype of the host (Lambrechts *et al.*, 2006). Genotype-by-genotype interaction is
58 particularly apparent in the literature on compatibility between schistosomes and their snail
59 first intermediate hosts (Richards and Shade, 1987; Morand *et al.*, 1996; Webster *et al.*, 2004;
60 Theron and Cousteau, 2005). As an agent of human schistosomiasis, *Schistosoma mansoni*
61 and its new world snail host, *Biomphalaria glabrata*, have been the subjects of numerous
62 studies on the genetic basis of variation in compatibility between different laboratory strains
63 of snails and parasite (here we use the term “strain” to refer to a field isolate maintained in the
64 laboratory for at least one generation). One consistent result, in study after study, is that snail
65 strains that are naturally or selected to be highly incompatible with one particular schistosome
66 strain, often remain highly compatible with other schistosome strains (Richards and Shade,
67 1987; Webster and Woolhouse, 1998; Webster *et al.*, 2004). Susceptibility to particular
68 schistosome strains can be highly heritable in snails (Richards and Shade, 1987; Richards *et*
69 *al.*, 1992; Webster *et al.*, 2004). However, attempts to analyze the genetic basis of differences
70 among particular snail strains in their susceptibility to a given schistosome strain have
71 suggested a variety of mechanisms, including single or multi-locus inheritance, and either
72 dominance or recessiveness of the trait (Richards, 1975; Richards *et al.*, 1992; Webster 2001).
73 To date, only one candidate locus has been found to associate with compatibility in either
74 parasite or host (Goodall *et al.*, 2006; Bender *et al.*, 2007).

75

76 Here we present compatibility studies conducted using lab and field samples of snails and
77 schistosomes, all derived from the same natural population in Guadeloupe, West Indies. We
78 show that results of compatibility trials depend very much on whether one uses laboratory-
79 cultured or field-collected individuals. We lay out possible hypotheses to explain some of
80 these results in the hopes of stimulating new research directions.

81

82 MATERIALS AND METHODS

83 *Brief overview of experiments*

84 We tested snail-schistosome compatibility through four main types of experiments (table 1) :
85 (i) compatibility trails using lab strains of snails and schistosomes ; (ii) compatibility trails
86 using wild snails and wild schistosomes; (iii) compatibility trials involving first wild snails
87 and parasites and later their corresponding snails and schistosomes after each of the first three
88 generations of lab culture; finally (iv) compatibility trials using lab snails challenged by wild
89 schistosomes.

90

91 *Sampling site*

92 All host and parasite samples in this study originated from the same transmission site at Dans
93 Fond (DFO; N:16°18.500', W:061°30.720'), located in the marshy forest of Grande Terre
94 Island in Guadeloupe, West Indies (Theron and Pointier, 1995). This site has been the subject
95 of numerous studies on the ecology, dynamics and genetics of larval and adult *S. mansoni*,
96 their intermediate host, *B. glabrata*, and their definitive host, *Rattus rattus* (Sire *et al.*, 1999;
97 2001 *a,b*; Theron *et al.*, 2004; Prugnolle *et al.*, 2005*a,b*; 2006). Transmission is characterized
98 here by very low prevalence in snails (0.6% on average), contrasting with very high infection
99 rates (94%) and heavy schistosome loads (160 worms per rat on average) within the definitive
100 hosts.

101

102 *Measuring compatibility: snail exposure, infection rates and intensities*

103 The level of compatibility for a particular snail-schistosome combination is traditionnally
104 quantified as the proportion of snails infected after individual exposure to a fixed number of
105 miracidia, (usually in the range of 5-20 miracidia). Snail infection rates vary with the parasite
106 dose used (Theron *et al.*, 1997), which makes it difficult to compare the outcomes of different
107 studies. Therefore, we evaluated compatibility between host and parasite populations or
108 strains by challenging individual snails with different numbers of miracidia. Although such
109 dose-response curves are labor-intensive to produce, they are much more informative about
110 the dynamics of compatibility between two strains than are single-dose challenges. All
111 challenge experiments described in this study were conducted by the same person (A.
112 Rognon), using the same protocols. For each experiment, snails (10-12 mm in diameter) were
113 exposed individually to a fixed number of miracidia in approximately 10 ml of water for 8
114 hours. Following exposure to miracidia, snails were replaced in their original containers until
115 their infection status was assessed.

116

117 The infected or uninfected status of the exposed snails was detected by two different
118 methods : (i) the shedding of cercariae 30 days post-exposure (the length of the prepatent
119 period); (ii) the presence of well developed mother sporocysts (M_{Sp}) in the head foot region
120 15 days post exposure. For the detection of mother sporocysts, the snails were fixed 15 days
121 post-exposure following the methods described by Theron and Gerard (1994). In brief, snails
122 were relaxed in pond water containing an excess crystalline menthol for 12 hours. The snail
123 body was removed and fixed in modified Raillet-Henry's solution. The number of M_{Sp}
124 present in each snail was determined following exhaustive dissection of the head-foot zone. In
125 this technique, M_{Sp}'s were readily observable as translucent white bodies within an opaque
126 grey tissue background (Fig. 1). For field-collected snails, the technique also allowed us to
127 distinguish *a posteriori* between snails infected during the experiment from those naturally
128 infected but undetectable because they were in the prepatent period during sampling. This
129 distinction is easily made by the presence of young daughter sporocysts in the hepatopancreas
130 of the snails at the time of dissection.

131

132 *Compatibility trials using laboratory strains*

133 Laboratory strains of snails and of schistosomes have been isolated from Dans Fond and
134 established in our laboratory at the University of Perpignan three times: in 1992, 2000 and
135 2005 (Table 1).

136

137 The snail strains were founded each time using 100-150 uninfected founders from the field.
138 The snail populations were allowed to expand quickly and were then maintained at a census
139 size in the hundreds of individuals. Snails in the lab are allowed to breed freely, and the lab
140 populations are not exposed to any deliberate selection. Note that although *B. glabrata* are
141 hermaphroditic, they preferentially outcross and show Hardy-Weinberg equilibrium at
142 molecular markers in both laboratory and field populations (Prugnolle *et al.*, 2005*b*; unpub.
143 data).

144

145 The schistosomes strains were founded each time using cercariae from 43-47 infected snails
146 previously exposed to 20 miracidia hatched from eggs collected from the livers of six
147 naturally-infected *R. Rattus*. In the lab, schistosome strains are passaged each generation
148 through five mice and approximately 24 infected snails (8-10 miracidia at exposure).

149

150 Compatibility trials were performed for snail and parasite strains established in 1992 and
151 2005 and tested after 5 years (Theron *et al.*, 1997) and 2 years maintenance in the lab,
152 respectively (labelled as “LAB 1997” and “LAB 2007” in Fig. 2A). Dose-response curves
153 were obtained by challenging individual snails (45-50 snails per treatment) with doses of 1, 2,
154 5, 10, 20, 30 and 50 miracidia. The doses of 20 and 5 miracidia were omitted for the LAB
155 1997 and LAB 2007 experiments, respectively. For both experiments snail infections rates
156 and parasite intensities were evaluated by mother sporocyst count.

157

158 *Compatibility trials using wild schistosomes and wild snails*

159 In 2000 and 2005 we generated dose-response curves using schistosomes and snails collected
160 directly from the transmission site (i.e. never passaged in the lab). We refer to these
161 experiments as “DFO 2000” and “DFO 2005”. All challenges were conducted at the INRA-
162 Duclos laboratory in Guadeloupe. The livers of heavily infected rats were crushed in a saline
163 solution, and the homogenates were passed through different filters to finally retrieve only
164 schistosome eggs. For each experiment, schistosome eggs were collected from six heavily
165 infected rats (> 100 worms/host) and placed in fresh water to hatch into miracidia.

166

167 Dose-response curves were obtained by challenging individual snails (50 snails per treatment)
168 with doses of 1, 2, 10, 20, 30 and 50 miracidia (Fig. 2B). For the DFO 2000 experiment, all
169 the snails were fixed 15 days post exposure and dissected to count numbers of mother
170 sporocysts per snail. Dissections showed no evidence of pre-patent infections in any of our
171 field-collected snails. Also, the prevalence of infection in the field averages only 0.6%.
172 Therefore we used the less-laborious cercarial shedding test for the DFO 2005 experiment on
173 the assumption that pre-patent infections would be so rare as to not appreciably influence our
174 results.

175

176 *Compatibility during the first three generations of laboratory passage*

177 In this experiment we followed the change in compatibility between snails and parasites in
178 going from the field through the first three generations of passage in the laboratory. Here we
179 used a single challenge dose (20 miracidia), rather than generating a dose-response curve each
180 time. In 2000, one hundred field-collected snails were individually exposed to 20 wild
181 miracidia. Fifty of these snails were transported back to our laboratory in France, and
182 cercariae shed by 43 of the 46 survivors were used to infect mice and establish a schistosome
183 strain. The remaining 50 wild exposed snails were fixed and dissected to measure infection

184 rates and numbers of mother sporocysts per snail. The lab strain of snails was established
185 from 100 uninfected, field-collected founders. After each of the first three generations of
186 culture in France, 50 lab snails were each challenged with 20 lab miracidia. These were also
187 fixed and dissected to measure infection rates and numbers of mother sporocysts per snail.

188

189 The lab strains isolated in 2005 were tested in the field, and then after each generation of
190 parasite passage, using a dose of 20 miracidia. We have continued testing them each
191 generation, using the same dose.

192

193 *Compatibility trials using wild versus laboratory snails or parasites*

194 It would be ideal to also have replicated trials in which lab parasites were used to challenge
195 field snails, and field parasites used to challenge lab snails. Owing to logistical constraints the
196 only combination we were able to attempt was a single trial in which lab snails were
197 challenged with field parasites. After the laboratory strain of snail established in 2000 had
198 been in captivity for two years, 300 of these lab snails were transported to Guadeloupe to be
199 challenged with wild miracidia. We generated a dose-response curve using these snails and
200 the same protocols as for the wild-by-wild combinations. Infection rates and intensities
201 (number of mother sporocysts per snail) were measured by dissecting each snail. Because we
202 have not been able to replicate this experiment, we consider the results of this trial to be
203 preliminary. We present them here simply for the sake of completeness.

204

205 RESULTS

206

207 *Laboratory schistosomes versus laboratory snails*

208 The dose-response curve generated using the 1992 laboratory strains shows a rapid increase in
209 infection rate up to a dose of 10-20 miracidia, but then levels off at about 65% infection (LAB
210 1997 in Fig. 2A). This result suggests that about 35% of the snails in the laboratory
211 population were completely resistant to infection, regardless of the number of miracidia used
212 to challenge. A very similar result was obtained with the 2005 lab isolates, except the curve
213 leveled off at about 45% infection (LAB 2007 in Fig. 2A).

214

215 *Wild schistosomes versus wild snails*

216 Results obtained for the two wild-by-wild dose-response curves (experiments DFO 2000 and
217 DFO 2005) are shown in Fig. 2B. Infection rates increased rapidly with increasing dose,
218 reaching 90-100% at 20-30 miracidia, and 100% at the highest dose.

219

220 *Change in compatibility in going from field isolates to laboratory strains*

221 Among the 50 snails that were each exposed to 20 miracidia in Guadeloupe in 2000, 46
222 survived and 43 of those became infected (93.5%), shed cercariae, and were used to found the
223 schistosome colony in our lab in France. Among the 50 other snails exposed in the same
224 conditions and fixed, 48 harbored developed MSp's (96%). The compatibility of this pair of
225 strains using a dose of 20 miracidia dropped to 51.0%, 59.8% and 54.1 % after 1, 2, and 3
226 passages through laboratory mice, respectively (Fig. 3). A similar pattern occurred with the
227 strains isolated in 2005. Again, infection rates dropped from near 100% when wild snails
228 were challenged with 20 wild miracidia apiece, to around 40% after a single generation of
229 passage in the lab. We continue to check the infection rate after each passage, and it remains
230 at about 40% to this day (mean \pm 1 s.e. = 41.2 ± 2.8) – right in line with the results of the
231 dose-response curve (Fig. 2A).

232

233 *Wild schistosomes versus laboratory snails*

234 Infection rates of laboratory bred snails exposed to wild miracidia increased gradually to 95%
235 at the highest dose (Fig. 2C). Interestingly, the shape of this curve differs from those of the
236 wild-by-wild or lab-by-lab curves. Rather than quickly reaching a plateau, the infection rate
237 increases almost linearly. So this result seems intermediate between those of the lab-by-lab
238 and field-by-field trials. In this case lab snails appeared to be much more susceptible to
239 groups of wild miracidia than to the same number of lab miracidia, yet not as susceptible as
240 wild snails.

241

242 *Infection intensities (establishment of mother sporocysts)*

243 In the lab-by-lab trials, infection intensities rose gradually and then leveled off at
244 approximately 2-3 /snail after challenge by 10 or more miracidia (Fig. 2A). In contrast, in the
245 wild-by-wild challenge the mean number of mother sporocysts (MSp) that developed within
246 infected snails increased steadily to an average of 6.6 ± 1.3 MSp/snail at the 50 miracidial
247 dose (Fig. 2B), with a maximum of 16 MSp found in one snail. Infection intensities in the
248 wild-schistosome by lab-snail trials (Fig. 2C) were lower than in the field-by-field trials,
249 reaching a maximum of 3.4 MSp/snail at a miracidial dose of 50.

250 Figure 3 shows the change in number of MSp's per infected snail during the first three
251 generations of passage of the 2000 lab strains. Although a dose of 20 wild miracidia
252 produced an average of 5.37 MSp per infected snail in Guadeloupe, that number dropped to
253 2.04, 1.92 and 1.63 MSp/snail over three generations of passage in the lab. Thus, as with the
254 percentage of snails infected, the number of sporocysts per infected snail dropped
255 precipitously after the first generation of passage, and then remained relatively constant
256 thereafter.

257

258 DISCUSSION

259 *Loss of compatibility in going from field to lab*

260 In the year-2000 field trials a challenge with 20 wild miracidia produced 95% infection. Yet
261 after a single generation of passage in the lab (of schistosome through the new lab snails) the
262 percent infection dropped to 51% and remained between 54% and 60% for the next two
263 generations. The same result was obtained when we established new strains of parasite and
264 snail in 2005 – almost 100% infection in the field dropped to about 40% after a single passage
265 in lab, and stayed the same thereafter. The number of MSp established per infected snail also
266 drops after laboratory passage (Fig. 3).

267

268 Thus it appears that in three independent isolations of schistosomes and snails from the same
269 site, there was a massive drop in compatibility in going from field to lab. Even more
270 intriguing is the observation that in two independent isolations of snails and parasites from the
271 field, it appears that a large fraction of the lab snails became completely resistant to the lab
272 strain of schistosomes (Fig. 2A). In stark contrast, we see 100% compatibility when wild
273 snails are challenged with enough wild miracidia (above 20- 30 miracidia), and much higher
274 rates and intensities of infection at the lower doses (Fig. 2B).

275

276 There are three main hypotheses to explain these results. (1) Experimental artifact: the lab-vs-
277 lab trials were conducted in France, and the field-by-field trials were conducted in
278 Guadeloupe. Thus some experimental factor might have been consistently different between
279 the two settings. (2) Environmental effects: something about the laboratory environment
280 makes the parasite less infective and/or the snails more resistant. (2) Genetic effects: either
281 the snail population, the parasite population, or both underwent genetic changes during
282 laboratory culture.

283

284 *Experimental artifact*

285 We took great pains to insure that the trials conducted in the lab in Guadeloupe were as
286 similar as possible to the trials conducted in the lab in France (same person did all the trials,
287 same equipment, and so on). Regardless, it is difficult to imagine how experimental artifact
288 could produce the plateaus seen in the lab-by-lab curves, which suggest the existence of a
289 completely resistant subset of the lab snail population. For example, if some factor (say the
290 water) reduced the average infectivity of miracidia in the lab in France (or resistance of the
291 snails), then the lab-by-lab curves would have shapes similar to those of the field-by-field
292 curves, but simply with a lower inflection point. Thus, we think that the fundamentally
293 different shapes of the two sets of curves really does reveal an interesting biological
294 phenomenon that deserves further study.

295

296 *Environmental effects*

297 It is well known that the susceptibility of snails can depend on, for example, their size, age
298 and physiological status (Anderson *et al.*, 1982; Theron *et al.*, 1998; Krist *et al.*, 2004). So it
299 is quite plausible that something about being raised in the lab environment makes snails more
300 resistant. For example, perhaps nutritional status differs or some microbe or other stressor in
301 the lab tank keeps the immune system of lab snails ramped up (Hertel *et al.* 2002). But again,
302 it is difficult to imagine a mechanism that would generate a completely resistant subset of the
303 population, rather than just a higher average resistance per snail. An environmental effect on
304 the parasite is also possible, although again we do not have any candidate mechanism that
305 would render that strain of parasites completely unable to infect just a subset of the snail
306 population.

307

308 *Genetic effects*

309 (1) Genetic change in the snails: The lab breeding colony of snails is never challenged, so any
310 selected change must have been a correlated response to selection on some other trait that is
311 inadvertently under selection in the lab. Genetic drift remains a possibility, but the lab colony
312 was founded each time using more than 100 individuals, and remained large thereafter. Also,
313 lab strains of *B. glabrata* tend to retain substantial molecular genetic diversity (e.g. Mulvey
314 and Vrijenhoek, 1981; Campos *et al.*, 2002). Thus, it is hard to believe that just one or two
315 generations of drift in the snails caused the changes we observed (e.g. Fig. 3).

316

317 (2) Genetic change in the schistosomes: If selection is involved, one possible source is the
318 definitive host. Rats are the definitive host in nature, but the parasites are passed through
319 mice in the lab. It would be quite interesting if selection for performance in a novel definitive
320 host resulted in a correlated response in infectivity to snails. Such a result would suggest that
321 the parasite uses some common mechanism to infect both the intermediate and definitive
322 hosts.

323

324 An even more plausible genetic explanation is rapid genetic drift in the lab schistosome
325 isolate. The miracidium that infects a snail becomes a sporocyst that then undergoes a round
326 of asexual reproduction that culminates in the release of thousands of genetically identical
327 cercariae. In most labs, rodents are infected by being placed in water that contains cercariae
328 shed by several infected snails. Thus, there is a huge potential for non-random variance
329 among clones in transmission to the next generation if care is not taken to equalize the
330 cercarial contribution from each snail. Strong selection among clones in going from the
331 natural definitive host (rats or humans) to mice or hamsters in the lab would add an
332 additional, non-random component to the among-clone variance in transmission success (e.g.
333 we see high variance among clones in their inherent infectivity to mice; J. Boissier, pers.
334 comm.). Rodents are expensive to maintain, so most lab strains of *S. mansoni* are passed
335 through only a handful of rodents each generation. A mouse can support 50-100 adult
336 schistosomes at most. We have little idea what is the variance in family size (egg production)
337 among adult worms, and thus what is the effective number of breeders per host that
338 contributes to the next generation (Criscione and Blouin, 2005). Thus, most lab strains of
339 schistosomes probably undergo severe genetic bottlenecks beginning with the first generation
340 of lab passage. Consistent with this prediction, molecular genetic studies show that lab strains
341 of schistosomes have only a fraction of the allelic variation found in field samples, and they
342 also show the distorted allele frequency distributions typical of bottlenecked populations
343 (Cornuet and Luikart, 1997; Stohler *et al.*, 2004).

344

345 *A possible mechanism: matching alleles and bottlenecks*

346 One hypothesis that would explain our results is that the success or failure of a challenge by
347 one parasite depends on the matched/mismatched status of the host and parasite genotypes, as
348 in matching-alleles models (Agrawal and Lively, 2002; Basch, 1975; Theron and Coustau,
349 2005). Any one snail and miracidium may have a modest probability of matching. However,
350 as a snail is challenged with increasing numbers of miracidia, the probability of at least one

351 match increases rapidly. Increasing the miracidial dose simply involves sampling a larger
 352 fraction of the phenotypic diversity in the parasite population. Under this model all snails
 353 eventually succumb when challenged by enough wild miracidia because the probability of at
 354 least one match approached 1.0. Such a system of phenotype matching could then explain the
 355 drop in compatibility after laboratory passage if the phenotypic variation is genetically based.
 356 If laboratory passage involves a more severe genetic bottleneck and loss of alleles in the
 357 parasite population than in the snail population, then a large fraction of the snails could
 358 quickly become “resistant”, owing simply to loss of compatible alleles in the schistosome
 359 strain. Under this scenario those snails are not “resistant” to schistosomes *per se*. They are
 360 simply not matched by any individuals in the now genetically-depauperate lab strain of
 361 parasites.

362

363 Such a genetically-based system of phenotype matching could generate curves that look very
 364 much like those in figure 2A and 2B. If each miracidium has a low, but constant probability
 365 of matching a target snail, and if miracidia infect independently of each other, then the
 366 probability that a snail becomes infected when challenged by x miracidia can be written

367

368 (1) $P(\text{infection}) = \left(1 - (1 - p)^x\right),$

369

370 where p = the probability of infection per miracidium. For example, in figure 4A we plotted
 371 equation 1 with a value of $p = 0.11$ in the same panel as the wild-vs.-wild data from 2002.
 372 Curves like those in Fig. 2B, in which there appears to be a completely resistant subset of
 373 snails, can be generated simply by multiplying equation 1 by a constant, f , which is the
 374 fraction of snails that can be infected. For example, in figure 4B we plotted the 1997 lab-vs.-
 375 lab data and equation (1) with $f = 0.67$ and $p = 0.2$.

376

377 One can also generate similar-looking curves via a matching genotypes model. Here the snail
 378 becomes infected if at least one miracidium in the pool of challengers carries the same
 379 (matching) genotype as the snail. The probability that a snail becomes infected when
 380 challenged by x miracidia equals

381

382 (2) $P(\text{infection}) = f \sum_{i=1}^{N_a} (h_i)(1 - (1 - p_i)^x) \quad ,$

383

384 where N_a = the number of genotypes present in the parasite population, h_i = the frequency of
385 genotype i in the host population, p_i = the frequency of genotype i in the parasite population.
386 If all snail alleles have a matching allele in the parasite population, then these curves will
387 asymptote to 100% infection as x , the number of challenging miracidia, increases. If some
388 snail genotypes are not matched by a genotype in the parasite population, then the curve will
389 asymptote at f , the fraction of snails that can be matched.

390

391 The actual shapes of curves generated by equation 2 will obviously depend on the particular
392 allele frequency distributions in parasite and host populations. We can, *a posteriori*, choose
393 parameter values that make the curves from equation 3 fit some of our observed data quite
394 well (Fig. 4). This does not mean we have proof for a matching genotypes model, but it does
395 show that such a model remains a plausible explanation for our results.

396

397 *Some possible consequences of using bottlenecked lab strains*

398 If the bottleneck hypothesis is correct, then this could explain why studies to date show such a
399 variety of genetic mechanisms and of strain-by-strain interactions. The chance loss or fixation
400 of alleles at compatibility loci may cause different loci to appear overly important in
401 controlling compatibility in different pair-wise combinations. In other words, in different
402 studies a different set of loci may, by chance, explain a disproportionate amount of the
403 variance in compatibility between particular lab strains. Loci that might be particularly
404 misleading in that sense include loci encoding highly polymorphic systems of matching
405 alleles that hosts use to recognize invaders, and/or invaders use to mimic hosts (Zhang *et al.*,
406 2004; Theron and Coustau 2005). Here the compatibility of any pair-wise combination could
407 depend largely on which matching alleles were lost by chance during the domestication of
408 each partner, and so would not predict compatibility with other strains, or with the original
409 field populations. For example, this hypothesis predicts that if we had selected the apparently
410 resistant fraction of snails revealed in Figure 2A, we would have rapidly obtained a snail
411 strain that is highly “resistant” to that particular schistosome strain (we are currently
412 conducting that experiment using the 2005 strains). However, in this case the proportion of
413 snails that did not acquire infection would not be *sensus stricto* resistant to *S. mansoni*. They
414 would simply be not matched by the reduced number of parasite genotypes contained within
415 that particular bottlenecked laboratory strain of schistosome. Those snails might remain

416 highly compatible with other schistosome strains that retain different alleles (*cf* Theron &
417 Coustau, 2005).

418

419 We are not arguing that all variation in compatibility results from polymorphic systems of
420 matching alleles. Compatibility is a complex process that involves many steps from
421 recognizing the invader to preventing its successful establishment (Loker *et al.*, 2004).

422 Indeed, the only resistance locus identified to date (Goodall *et al.*, 2006; Bender *et al.*, 2007)
423 is clearly part of an effector mechanism – killing the parasite once it has been successfully
424 recognized by the host. But the idea that using inbred strains causes different loci to be
425 important in each strain-by-strain comparison would hold for any polymorphic, genetically-
426 based mechanism of resistance.

427

428 *Some caveats*

429 We recognize that replicated trials of each reciprocal combination of field-by-lab would have
430 gone a long way towards identifying which species is responsible for the difference between
431 lab and field trials. The one combination we were able to attempt (lab-snails versus field-
432 schistosomes) produced intriguing results in that infection rates were, overall, lower than in
433 the wild-by-wild combination, but there was no plateau. The higher infection rates are
434 consistent with the bottleneck hypothesis. But if parasite diversity was all that mattered, the
435 curve should have looked just like the field-by-field curves, not the more gradual increase
436 with dose that we observed. Taken at face value, this result suggests that something about the
437 snails also changed in the lab. But until this result can be replicated, we hesitate to make too
438 much of it.

439

440 *Summary*

441 To our knowledge, this is the first study to compare the results of compatibility trials using
442 lab-reared and field-collected individuals from the same source populations. We showed that
443 even a single generation of laboratory passage had a large effect on the results of
444 compatibility trials. Barring experimental artifact, possible explanations include
445 environmental effects on either host or parasite, and genetic change in either host or parasite.
446 We suggest one possible mechanism that could produce curves very much like those
447 observed. The bottleneck hypothesis could explain much of the highly variable strain-by-
448 strain compatibilities that are so apparent in the literature on schistosomes and their snail
449 hosts.

450

451 ACKNOWLEDGEMENTS

452 We thank Hervé Mauleon (INRA, Domaine Duclos) for logistical support in Guadeloupe, and
453 Jean-Pierre Pointier for help with field samples. This work received financial support from
454 the CNRS (EDD), and the Ministère de l'Ecologie et du Développement Durable (Ecosystèmes
455 Tropicaux n° 752600). Thanks to C. Bayne, J. Tennessen, C. Adema and M. Steinauer for
456 comments on earlier drafts. M. Blouin's sabbatical at CNRS-UPVD was supported by the
457 University of Perpignan and Oregon State University.

458 REFERENCES

459

- 460 **Agrawal, A. F. and Lively, C. M.** (2003). Modelling infection as a two-step process
461 combining gene-for-gene and matching-allele genetics. *Proceedings. Biological sciences /*
462 *The Royal Society* **270**, 323-334.
- 463 **Anderson, R. M., Mercer, J. G., Wilson, R. A. and Carter, N. P.** (1982). Transmission of
464 *Schistosoma mansoni* from man to snail: experimental studies of miracidial survival and
465 infectivity in relation to larval age, water temperature, host size and host age. *Parasitology* **85**
466 **(Pt 2)**, 339-360.
- 467 **Basch, P. F.** (1975). An interpretation of snail-trematode infection rates: specificity based
468 on concordance of compatible phenotypes. *International Journal for Parasitology* **5**, 449-452.
- 469 **Bayne, C. J., Hahn, U. K. and Bender, R. C.** (2001). Mechanisms of molluscan host
470 resistance and of parasite strategies for survival. *Parasitology* **123 Suppl**, S159-167.
- 471 **Campos, Y. R., Carvalho, O. S., Goveia, C. O. and Romanha, A. J.** (2002). Genetic
472 variability of the main intermediate host of the *Schistosoma mansoni* in Brazil, *Biomphalaria*
473 *glabrata* (Gastropoda: Planorbidae) assessed by SSR-PCR. *Acta Tropica* **83**, 19-27.
- 474 **Cornuet, J. M. and Luikart, G.** (1996). Description and power analysis of two tests for
475 detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.
- 476 **Criscione, C. D. and Blouin, M. S.** (2005). Effective sizes of macroparasite populations: a
477 conceptual model. *Trends in Parasitology* **21**, 212-217.
- 478 **Goodall, C. P., Bender, R. C., Brooks, J. K. and Bayne, C. J.** (2006). *Biomphalaria*
479 *glabrata* cytosolic copper/zinc superoxide dismutase (SOD1) gene: association of SOD1
480 alleles with resistance/susceptibility to *Schistosoma mansoni*. *Molecular and Biochemical*
481 *Parasitology* **147**, 207-210.
- 482 **Hertel, L. A., Bayne, C. J. and Loker, E. S.** (2002). The symbiont *Capsaspora owczarzaki*,
483 nov. gen. nov. sp., isolated from three strains of the pulmonate snail *Biomphalaria glabrata* is
484 related to members of the Mesomycetozoa. *International Journal for Parasitology* **32**, 1183-
485 1191.
- 486 **Krist, A. C., Jokela, J., Wiehn, J. and Lively, C. M.** (2004). Effects of host condition on
487 susceptibility to infection, parasite developmental rate, and parasite transmission in a snail-
488 trematode interaction. *Journal of Evolutionary Biology* **17**, 33-40.
- 489 **Lambrechts, L., Fellous, S. and Koella, J. C.** (2006). Coevolutionary interactions between
490 host and parasite genotypes. *Trends in Parasitology* **22**, 12-16.

491 **Loker, E. S., Adema, C. M., Zhang, S. M. and Kepler, T. B.** (2004). Invertebrate immune
492 systems--not homogeneous, not simple, not well understood. *Immunological Reviews* **198**, 10-
493 24.

494 **Morand, S., Manning, S. D. and Woolhouse, M. E.** (1996). Parasite-host coevolution and
495 geographic patterns of parasite infectivity and host susceptibility. *Proceedings. Biological*
496 *sciences / The Royal Society* **263**, 119-128.

497 **Mulvey, M. and Vrijenhoek, R. C.** (1981). Genetic variation among laboratory strains of the
498 planorbid snail *Biomphalaria glabrata*. *Biochemical Genetics* **19**, 1169-1182.

499 **Prugnolle, F., De Meeus, T., Pointier, J. P., Durand, P., Rognon, A. and Theron, A.**
500 (2006). Geographical variations in infectivity and susceptibility in the host-parasite system
501 *Schistosoma mansoni/Biomphalaria glabrata*: no evidence for local adaptation. *Parasitology*
502 **133**, 313-319.

503 **Prugnolle, F., Liu, H., De Meeus, T. and Balloux, F.** (2005a). Population genetics of
504 complex life-cycle parasites: an illustration with trematodes. *International Journal For*
505 *Parasitology* **35**, 255-263.

506 **Prugnolle, F., Theron, A., Pointier, J. P., Jabbour-Zahab, R., Jarne, P., Durand, P. and**
507 **De Meeus, T.** (2005b). Dispersal in a parasitic worm and its two hosts: consequence for local
508 adaptation. *Evolution Int J Org Evolution* **59**, 296-303.

509 **Richards, C. S.** (1975). Genetic factors in susceptibility of *Biomphalaria glabrata* for
510 different strains of *Schistosoma mansoni*. *Parasitology* **70**, 231-241.

511 **Richards, C. S., Knight, M. and Lewis, F. A.** (1992). Genetics of *Biomphalaria glabrata*
512 and its effect on the outcome of *Schistosoma mansoni* infection. *Parasitology Today* **8**, 171-
513 174.

514 **Richards, C. S. and Shade, P. C.** (1987). The genetic variation of compatibility in
515 *Biomphalaria glabrata* and *Schistosoma mansoni*. *Journal of Parasitol* **73**, 1146-1151.

516 **Sire, C., Durand, P., Pointier, J. P. and Theron, A.** (1999). Genetic diversity and
517 recruitment pattern of *Schistosoma mansoni* in a *Biomphalaria glabrata* snail population: a
518 field study using random-amplified polymorphic DNA markers. *Journal of Parasitol* **85**, 436-
519 441.

520 **Sire, C., Durand, P., Pointier, J. P. and Theron, A.** (2001a). Genetic diversity of
521 *Schistosoma mansoni* within and among individual hosts (*Rattus rattus*): infrapopulation
522 differentiation at microspatial scale. *International Journal for Parasitology* **31**, 1609-1616.

523 **Sire, C., Langand, J., Barral, V. and Theron, A.** (2001b). Parasite (*Schistosoma mansoni*)
524 and host (*Biomphalaria glabrata*) genetic diversity: population structure in a fragmented
525 landscape. *Parasitology* **122**, 545-554.

526 **Stohler, R. A., Curtis, J. and Minchella, D. J.** (2004). A comparison of microsatellite
527 polymorphism and heterozygosity among field and laboratory populations of *Schistosoma*
528 *mansoni*. *International Journal for Parasitology* **34**, 595-601.

529 **Theron, A. and Coustau, C.** (2005). Are *Biomphalaria* snails resistant to *Schistosoma*
530 *mansoni*? *Journal of Helminthology* **79**, 187-191.

531 **Theron, A. and Gerard, C.** (1994). Development of accessory sexual organs in *Biomphalaria*
532 *glabrata* as related to infection timing by *Schistosoma mansoni*: Consequences on the energy
533 utilisation patterns by the parasite. *The Journal of Molluscan Studies* **60**, 78-85.

534 **Theron, A., Pages, J. R. and Rognon, A.** (1997). *Schistosoma mansoni*: distribution patterns
535 of miracidia among *Biomphalaria glabrata* snail as related to host susceptibility and
536 sporocyst regulatory processes. *Experimental Parasitology* **85**, 1-9.

537 **Theron, A. and Pointier, J. P.** (1995). Ecology, dynamics, genetics and divergence of
538 trematode populations in heterogeneous environments: the model of *Schistosoma mansoni* in
539 the insular focus of Guadeloupe. *Research Reviews in Parasitology* **55**, 49-64.

540 **Theron, A., Rognon, A. and Pages, J. R.** (1998). Host choice by larval parasites: a study of
541 *Biomphalaria glabrata* snails and *Schistosoma mansoni* miracidia related to host size.
542 *Parasitology Research* **84**, 727-732.

543 **Theron, A., Sire, C., Rognon, A., Prugnolle, F. and Durand, P.** (2004). Molecular ecology
544 of *Schistosoma mansoni* transmission inferred from the genetic composition of larval and
545 adult infrapopulations within intermediate and definitive hosts. *Parasitology* **129**, 571-585.

546 **Webster, J. P.** (2001). Compatibility and sex in a snail-schistosome system. *Parasitology*
547 **122**, 423-432.

548 **Webster, J. P., Gower, C. M. and Blair, L.** (2004). Do hosts and parasites coevolve?
549 Empirical support from the *Schistosoma* system. *American Naturalist* **164 Suppl 5**, S33-51.

550 **Webster, J. P., Woolhouse, M.E.J.** (1998). Selection and strain specificity of compatibility
551 between snail intermediate hosts and their parasitic schistosomes. *Evolution Int J Org*
552 *Evolution* **52**, 1627-1634.

553 **Zhang, S. M., Adema, C. M., Kepler, T. B. and Loker, E. S.** (2004). Diversification of Ig
554 superfamily genes in an invertebrate. *Science* **305**, 251-254.

555

556

Table 1. Summary of experiments conducted.

557

Strains compared	Year strains isolated from field	Year tested	Dose-response curve done?	Method used to verify infection ³
lab-vs-lab	1992	1997	Yes	Dissection
	2000	2000	No ¹	Dissection
	2005	2007	Yes ²	Dissection
wild-vs-wild	NA	2000	Yes	Dissection
	NA	2005	Yes	Shedding
wild parasites-vs-lab snails	2000 (snails)	2002	Yes	Dissection

558

559 ¹challenges using 20 miracidia performed for first three generations of laboratory passage.

560

561 ²challenges using 20 miracida performed every generation since the strains were established

562

563 ³snails were either fixed and dissected to count number of mother sporocysts (Dissection) or
564 were observed for cercarial shedding 30 days post exposure (Shedding).

565 **Figure legends**

566 Fig. 1. Head-foot region of a *Biomphalaria glabrata* fixed 15 days post-exposure to 20
567 miracidia of *Schistosoma mansoni*. Six well developed mother sporocysts of *Schistosoma*
568 *mansoni* are easily observable as white bodies within the snail tissue.

569
570 Fig. 2. Infection rates (\pm one standard error) of individual snails and mean number (bar \pm one
571 standard error) of mother sporocysts (M_{Sp}) per infected snail exposed to increasing doses of
572 *Schistosoma mansoni* miracidia (nMi). (A) Both parasites and snails were maintained in lab
573 for 5 years. N = 45 snails per dose. (B) Wild snails challenged with wild miracidia. Data
574 from 2 different trials in the DFO site. N = 39-50 surviving snails per dose. M_{Sp} data were
575 collected only in 2000. (C) Laboratory snails maintained for two years and then challenged
576 by wild miracidia. N = 39-45 surviving snails per dose.

577
578 Fig. 3. Snail infection rates (grey bar \pm one standard error) and mean number (black bar \pm
579 one standard error) of mother sporocysts (M_{Sp}) per infected snail after exposure to 20
580 miracidia. Snails and parasites were both obtained from the wild population (Field) in 2000,
581 and were then tested after 1, 2, and 3 laboratory generations of passage.

582
583 Fig. 4. Example of how simple models of independent infection with low individual infection
584 probabilities can generate curves that look very similar to some empirical curves. (A) 2000
585 wild-by-wild data (circles) versus equation 1 parameterized with $p = 0.11$ (line). (B) 1997
586 lab-by-lab data (circles) versus equation 2 parameterized by $f = 0.67$ and $p = 0.2$ (line).

587
588
589
590
591



592
593
594
595
596

597 **FIG. 1**
598

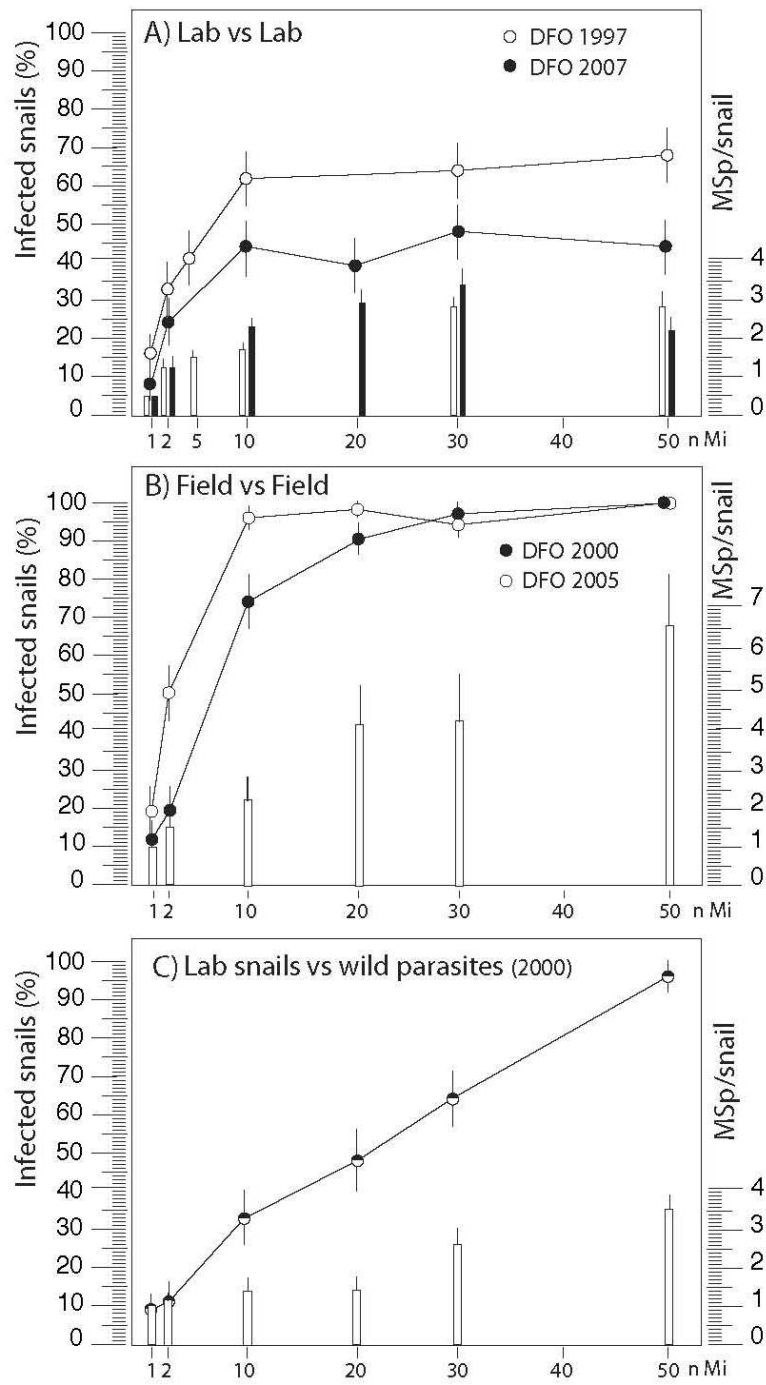


FIG.2

601
602
603
604

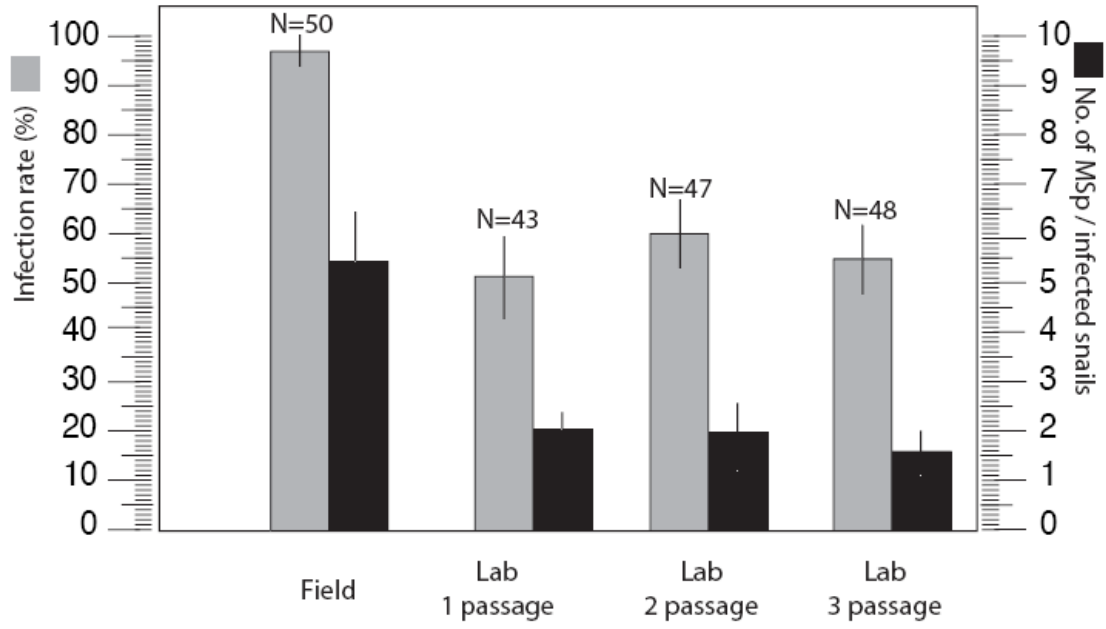


FIG.3

605
606
607

608
609
610
611

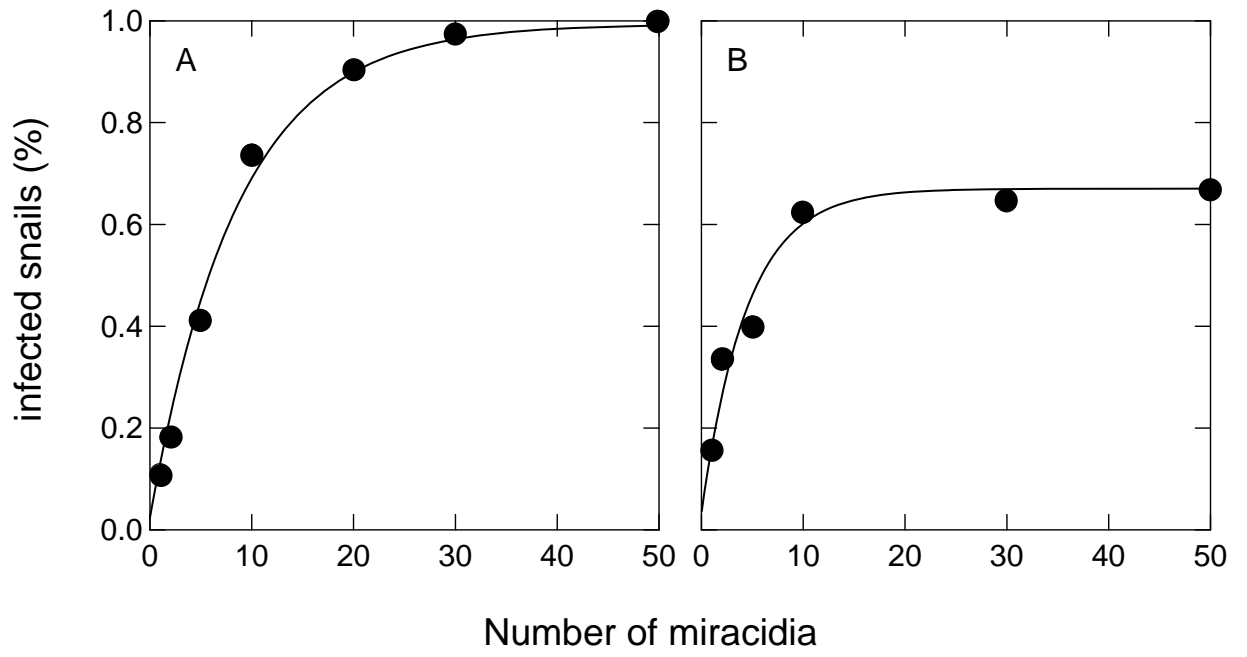


FIG. 4

612
613



FIG. 1

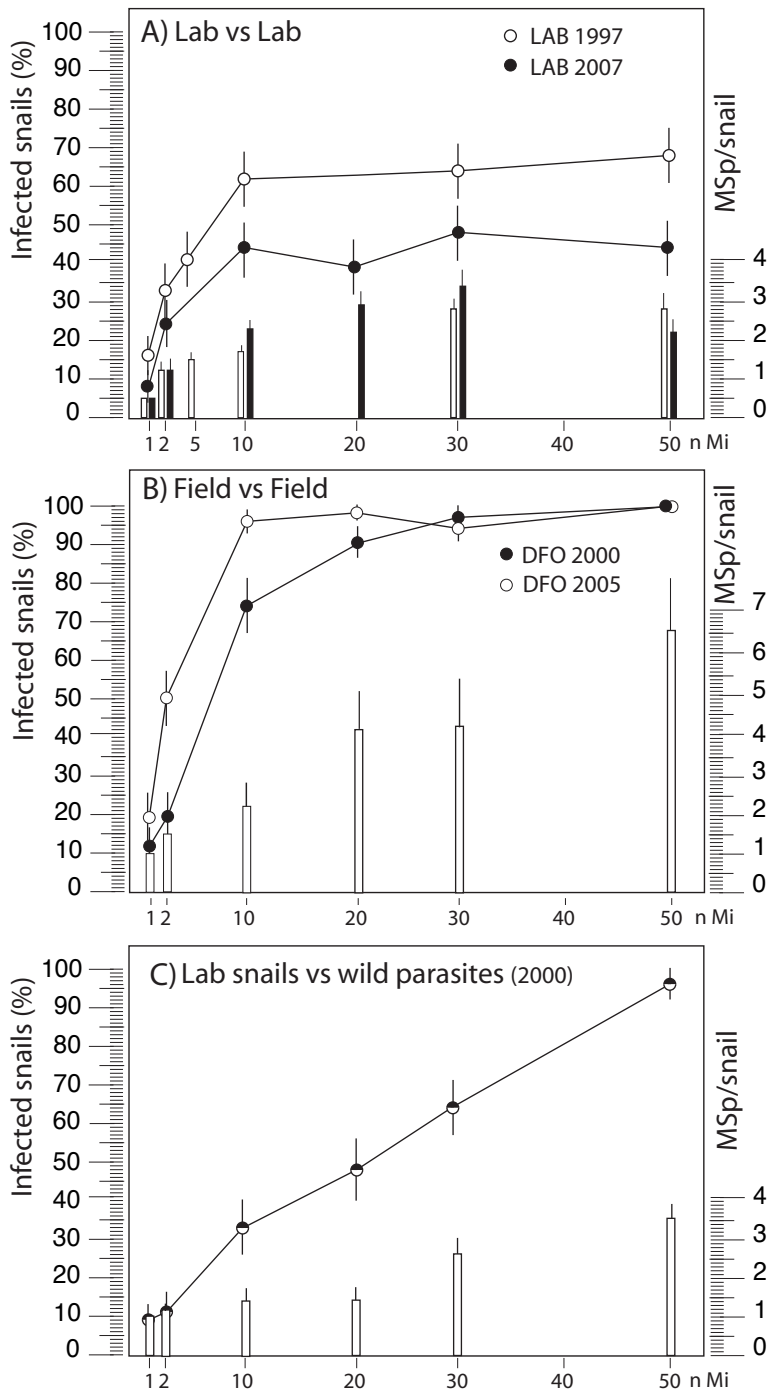


FIG. 2

MS ID: PAR-2008-0080.R1

Title: Effects of laboratory culture on compatibility between snails and schistosomes

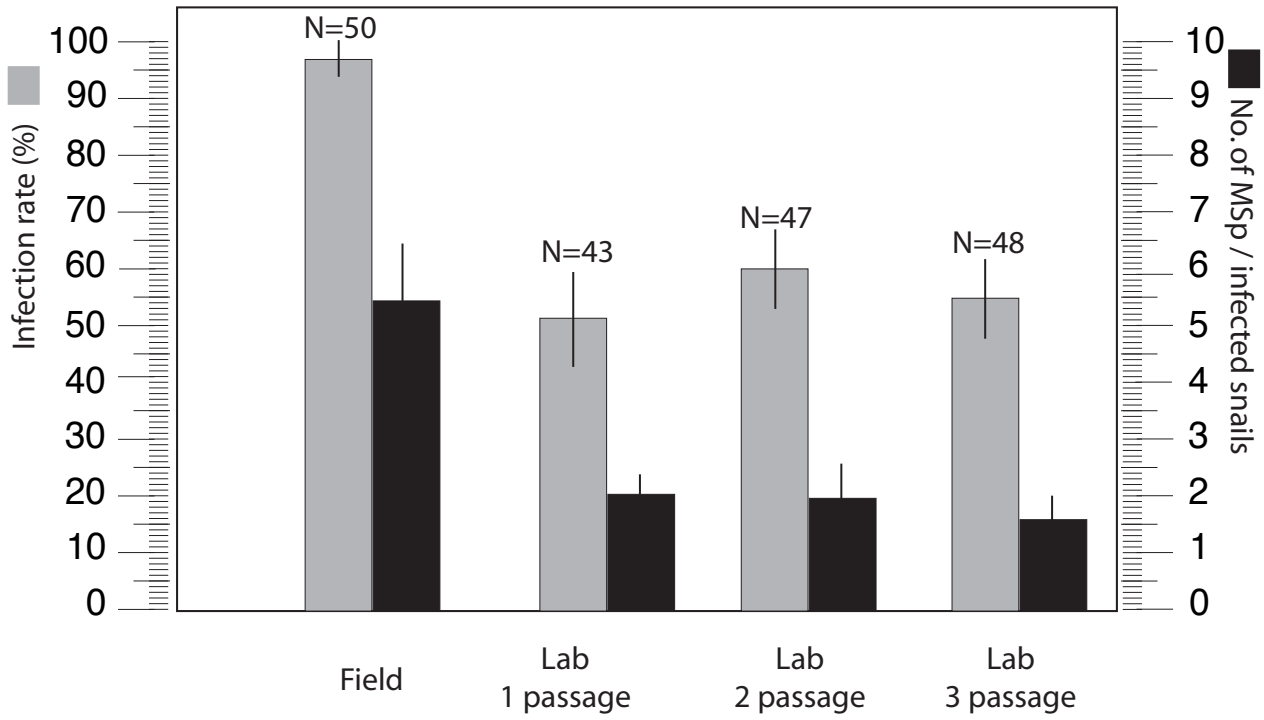


FIG.3

MS ID: PAR-2008-0080.R1

Title: Effects of laboratory culture on compatibility between snails and schistosomes

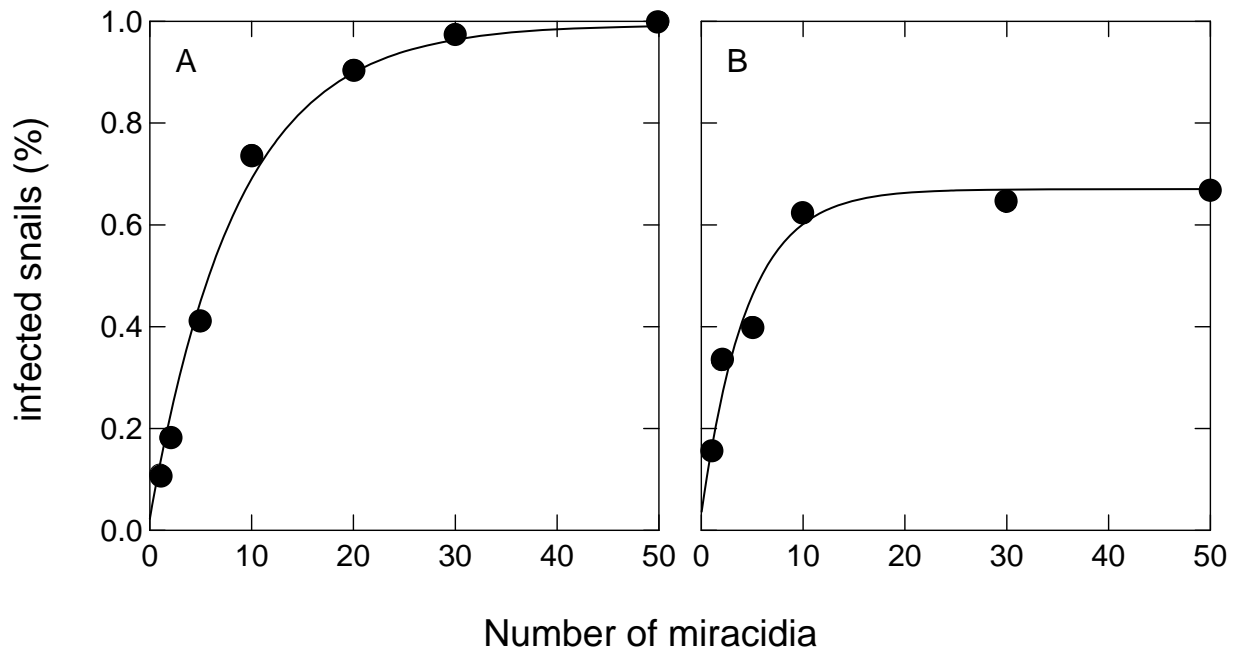


FIG. 4

MS ID: PAR-2008-0080.R1

Title: Effects of laboratory culture on compatibility between snails and schistosomes