



# Co-occurrences of parasite clones and altered host phenotype in a snail–trematode system

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## Abstract

The frequent co-occurrence of two or more genotypes of the same parasite species in the same individual hosts has often been predicted to select for higher levels of virulence. Thus, if parasites can adjust their level of host exploitation in response to competition for resources, mixed-clone infections should have more profound impacts on the host. Trematode parasites are known to induce a wide range of modifications in the morphology (size, shell shape or ornamentation) of their snail intermediate host. Still, whether mixed-clone trematode infections have additive effects on the phenotypic alterations of the host remains to be tested. Here, we used the snail *Potamopyrgus antipodarum*-infected by the trematode *Coitocaecum parvum* to test for both the general effect of the parasite on host phenotype and possible increased host exploitation in multi-clone infections. Significant differences in size, shell shape and spinosity were found between infected and uninfected snails, and we determined that one quarter of naturally infected snails supported mixed-clone infections of *C. parvum*. From the parasite perspective, this meant that almost half of the clones identified in this study shared their snail host with at least one other clone. Intra-host competition may be intense, with each clone in a mixed-clone infection experiencing major reductions in volume and number of sporocysts (and consequently multiplication rate and cercarial production) compared with single-clone infections. However, there was no significant difference in the intensity of host phenotype modifications between single and multiple-clone infections. These results demonstrate that competition between parasite genotypes may be strong, and suggest that the frequency of mixed-clone infections in this system may have selected for an increased level of host exploitation in the parasite population, such that a single-clone is associated with a high degree of host phenotypic alteration.

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## 1. Introduction

A feature of many natural parasite infections is the co-occurrence in the same host individual of two or more genotypes of the same parasite species (Read and Taylor, 2001; Gower and Webster, 2005; but see Sire et al., 1999 for contrasting results). This genetic diversity of natural infections provides one of the contexts in which parasites evolve. Many theoretical models predict that under a wide range of conditions, when two different parasite genotypes co-occur in the same host individual, within-host competi-

tion between them for the same resources will select for higher levels of host exploitation and/or virulence (Frank, 1992, 1996; May and Nowak, 1995; van Baalen and Sabelis, 1995; Read and Taylor, 2001; Gandon and Michalakis, 2002; Schjørring and Koella, 2003; but see Chao et al., 2000 for alternative predictions). Restraint by one genotype would be disadvantageous if another genotype is using up host resources and, thus, increased host exploitation appears to be the main solution to within-host selection acting on different parasites. Increased levels of host resources exploitation can become fixed in the parasite population if mixed infections are frequent generation after generation; otherwise, it may be expressed as a facultative strategy in response to competition for host resources.

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Empirical support for the models has come from studies on malaria, where two competing clonal lines in the same rodent host achieved higher rates of multiplication and transmission to mosquitos, and higher virulence to their rodent host, than single-clone infections (Taylor et al., 1997, 1998). The most virulent genotype consistently out-competes the less virulent one in mixed-genotype infections in the rodent (Bell et al., 2006) and achieves a higher transmission rate to mosquitoes (De Roode et al., 2005). However, studies on other host–parasite systems have provided contrasting results (see Imhoof and Schmid-Hempel, 1998) and further empirical research is necessary to assess the general role of genetic diversity as a determinant of parasite virulence.

Trematode parasites in their snail intermediate hosts present interesting model systems for examining the effects of parasite genetic diversity on host exploitation and/or virulence. Trematodes often cause the castration of their snail host (McClelland and Bourns, 1969; Baudoin, 1975; Sousa, 1983) and are also responsible for increased mortality of infected snails (Jokela et al., 1999; Fredensborg et al., 2005). In a study of single versus mixed-genotype infections involving a trematode, Davies et al. (2002) found that snails harbouring two laboratory strains of *Schistosoma mansoni* suffered greater reductions in fecundity and survival than snails infected with a single strain (but see Gower and Webster, 2005 for different scenarios). Trematodes can also modify the shell morphology of their hosts in quantifiable ways (Krist, 2000; McCarthy et al., 2004; Hay et al., 2005; Miura and Chiba, 2007). Altered shell shape induced by infection apparently serves to increase the volume available for parasite growth inside the shell (McCarthy et al., 2004). During competition for host resources by different trematode clones in mixed infections, available volume within the snail shell may become a key factor. Departures from the “normal” shell shape induced by trematodes could thus provide a simple but relevant measure of the variation in effects of infection on the host. However, alterations in host morphology from mixed versus single-clone infections may not represent parasite adaptations, but result from host-mediated alterations and indirect effects of multiple infections.

We investigated the impact of the trematode *Coitocaeum parvum* (Opecoelidae) on its first intermediate host, the snail *Potamopyrgus antipodarum* (see MacFarlane, 1939; Holton, 1984a,b for *C. parvum* life cycle details). Snails become infected when they are penetrated by miracidia, the small and ciliated infective stages hatched from the parasite’s eggs. Within the snail, a newly established miracidium multiplies asexually, forming sac-like sporocysts that will produce free-living infective stages (cercariae) responsible for the continuation of the parasite’s life cycle. After leaving the snail, cercariae penetrate and encyst in amphipods, to await ingestion by fish predators where they settle as adult egg-producing worms. The shell morphology of the snail *P. antipodarum* varies both within and among populations (Winterbourn, 1970), with individ-

uals ranging from smooth-shelled to very spiny. Recently, Levri et al. (2005) have shown that the morphology of *P. antipodarum* is sensitive to trematode infections, with snails infected by the trematode *Microphallus* sp. showing different length-to-width ratios and being less spiny than uninfected snails. It is these subtle changes in morphology caused by trematode infection that we use here as a measure of the impact of parasitism on the host.

Our study has three main objectives. Firstly, we determine if snails infected by the trematode *C. parvum* have altered shell features compared with uninfected snails. We focus on shell width as a function of length, spinosity, relative shell mass and volume within the shell available for parasite tissues. Second, we quantify the number of *C. parvum* clones per snail and the extent of competition for resources among these clones, by comparing sporocyst sizes and numbers between single-clone and mixed-clone infections. Each clone is issued from a different miracidium and clones can be distinguished using microsatellite loci (Lagrue et al., 2007). Third, we determine if mixed-clone infections lead to more pronounced alterations of shell features than single-clone infections. Additive effects of multiple parasite clones on shell shape could be a strategy serving to alleviate space limitations within the host. Altogether, our results provide insights into potential clonal interactions within the host, as well as interactions between infection and host features.

## 2. Materials and methods

### 2.1. Collection and processing of *Potamopyrgus antipodarum*

Snails were collected from macrophytes along the shoreline of Lake Waihola, South Island, New Zealand using dip nets in December 2005. The snails were kept alive in lake water before measurement and dissection. Prior to dissection, a digital picture of each snail was taken using an Olympus DP12 Microscope Digital Camera System connected to a stereo-microscope. Photographs were standardised by using a fixed magnification and focus so that the edge of the snail shell was sharp on the picture. For each photograph, the snail was oriented the same way with the shell opening facing up (Levri et al., 2005). Each snail was measured using the ImageJ software (Wayne Rasband, NIH, USA).

### 2.2. Shell shape and parasitism

From the initial collection, a sample of 1,000 snails was haphazardly chosen. Two initial measurements were taken for each snail (see Fig. 1): the total length of the shell and the width of the last grown whorl (whorl 1). Snails were also assessed for their degree of spinosity following the scale of 0 to 3 defined by Levri et al. (2005). A score of 0 indicated a smooth shell, a score of 1 indicated a shell with a ridge but no spines, a score of 2 was given to a shell with

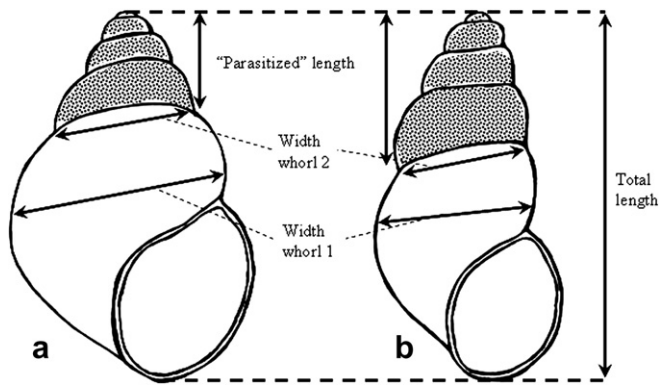


Fig. 1. Measurements of lengths and widths of the whole snail shell and of the part of the shell potentially available for the parasite (). (a) The typical shell shape of uninfected *Potamopyrgus antipodarum*. (b) The typical shell shape of *P. antipodarum* infected with *Coitocaecum parvum*.

short spines and a 3 indicated shells with fully developed spines.

Each snail was then dissected to determine whether or not it was infected by *C. parvum*. Snails were sorted by their infection status: uninfected or *C. parvum*-infected. Snails infected with other parasite species (i.e. *Microphallus* sp. ( $n = 6$ ; 0.6% prevalence) or unidentified trematode species ( $n = 3$ ; 0.3% prevalence)) were uncommon and were consequently excluded from the analyses. All 991 remaining snails were used to examine the effect of parasitism on shell size and shape. An analysis of variance (ANOVA) with snail class (uninfected or infected) as the main factor and total snail length as the dependent variable, and an analysis of covariance (ANCOVA) with snail class as the main factor, total length of the shell as a covariate and width of whorl 1 as the dependent variable, were performed to analyse these effects. Total length and width of whorl 1 were log-transformed before analyses to normalise their distributions.

The effect of parasitism on spinosity was examined by using a Chi-square test comparing the frequency of individuals in each spinosity class (0–3) between uninfected and infected snails. Previously, a one-way ANOVA was performed on the uninfected snail class, with length as the dependent variable and degree of spinosity as the main factor, to test for any association between snail size and the degree of spinosity. Since this analysis showed no effect of shell size on spinosity ( $F_{3,817} = 0.482$ ,  $P = 0.69$ ), all snail sizes were considered in the comparison between uninfected and infected snails.

A haphazardly chosen sub-sample of 262 snails ( $n = 131$  uninfected and  $n = 131$  *C. parvum*-infected) from the original sample of 991 snails was used to test for the effect of infection on shell volume. The snail shell was approximated by the shape of a cone (McCarthy et al., 2004) with a base diameter equivalent to the width of whorl 1 and of vertical height equal to the total length of the shell. The total volume of the shell was calculated using the formula  $V = 1/3(\pi r^2 h)$  where  $V$  is the volume,  $r$  the radius (half the width

of whorl 1) and  $h$  the vertical height (total length). *Coitocaecum parvum* castrates its host and uses the space in the shell usually occupied by the snail gonads (the posterior part of the shell from the second whorl up to the tip of the shell, see Fig. 1). Thus, the length of the posterior part of the shell (“parasitised” length) and the width of whorl 2 were measured and used to calculate the volume inside the snail shell potentially available for the parasite (i.e. “parasitised” shell, see Fig. 1). “Parasitised” volume was also approximated by the volume of a cone and calculated as above using the width of whorl 2 as base diameter and “parasitised” length as vertical height. The total shell volume and the “parasitised” shell volume were log-transformed before analyses. The effects of *C. parvum* infection on either shell volumes (total or “parasitised”) were tested using an ANCOVA with infection status as the main factor, total length of the shell as a covariate and each calculated shell volume as the dependent variable. A further ANCOVA was performed to test for the effect of infection on “parasitised” shell volume controlled for the total shell volume; here, snail class (uninfected or infected) was defined as the main factor, total shell volume as a covariate and “parasitised” shell volume as the dependent variable.

From the previous sub-sample of 232 snails, an equal number of uninfected ( $n = 50$ ) and infected ( $n = 50$ ) snails were used to test for the effect of parasitism on shell mass (i.e. thickness). Since we wanted to test for this effect on equivalent and therefore comparable shells, only snails with a spinosity degree of 0 were used. After being photographed and dissected, snails were dried overnight in an incubator and all remaining organic tissues were then burned for 2 h at 550 °C in a furnace. The dry shell was subsequently weighed to the nearest 0.01 mg and the result log-transformed before analyses. An ANCOVA was used to analyse the effect of *C. parvum* infection on shell mass with the infection status as main factor, total shell volume as a covariate and shell weight as the dependent variable.

### 2.3. Parasite clonal diversity, sporocyst volume and host shell morphology

To quantify the number of *C. parvum* clones per snail and examine relationships among parasite clonal diversity, parasite volume, number of sporocysts and host shell shape/volume, a new sample of snails from the initial collection was photographed, measured and dissected until 46 infected snails were found. After the snail dissection, the total number of sporocysts per snail was recorded, a photograph of these sporocysts was taken and the genotype of each sporocyst was then determined.

A total of 676 sporocysts were recovered from the 46 dissected snails, the mean number of sporocysts per snail was 14.7 ( $\pm 0.9$ ). All sporocysts were measured and their genotype determined. DNA was extracted by placing sporocysts individually in Eppendorf tubes with 400  $\mu$ l of 5% Chelex containing 0.1 mg/ml proteinase K, incubating at 60 °C

for 2 h and then 90 °C for 10 min. The genotype of each sporocyst was determined at eight microsatellite loci (Cpa-3, Cpa-4, Cpa-8, Cpa-12, Cpa-19, Cpa-26, Cpa-28 and Cpa-29) as described in Lagrue et al. (2007). The number of alleles per locus, expected and observed heterozygosities, deviations from Hardy–Weinberg expectations for each locus and across all loci,  $F_{IS}$  (Weir and Cockerham, 1984), and tests of genotypic disequilibrium between all pairs of loci were calculated with GENEPOP version 3.4 (Raymond and Rousset, 1995) using unique genotypes (identical genotypes are the result of asexual reproduction within snails). Significance of deviations from Hardy–Weinberg expectations was determined using the Markov chain exact probability test of Guo and Thompson (1992) and significance of linkage disequilibrium values was determined with Fisher's exact test as implemented in GENEPOP version 3.4 (both tests utilised 10,000 dememorisations, 1,000 batches and 10,000 iterations per batch). Alpha significance levels were corrected for multiple simultaneous pairwise comparisons using the sequential Bonferroni approach (Rice, 1989) for Hardy–Weinberg (initial  $\alpha = 0.0063$ ) and disequilibrium (initial  $\alpha = 0.0018$ ) analyses.

Sporocysts were approximated by the shape of a cylinder, the base of which equaled the diameter of the sporocyst and the height equaled the length of the sporocyst. The volume of each sporocyst was calculated using the formula  $V = \pi r^2 h$  where  $V$  is the volume,  $r$  the radius (half the diameter of the sporocyst) and  $h$  the vertical height (length of the sporocyst), allowing us to calculate the total volume of parasite and the mean sporocyst volume for each snail. The “parasitised” shell volume of the snails was calculated as mentioned above. The overall parasite volume and mean sporocyst volume as well as the total length, width of whorl 1 and “parasitised” shell volume of the snails were log-transformed before analyses. A one-way ANOVA using the number of clones as the main factor and total snail length as the dependent variable showed no differences in shell length between snails with different numbers of clones ( $F_{2,43} = 0.422$ ,  $P = 0.66$ ). Hence, the effects of the number of *C. parvum* clones on the overall parasite volume and mean sporocyst volume were tested using a one-way ANOVA with the number of clones as the main factor and either the overall parasite volume or mean sporocyst volume as dependent variables. A non-parametric Kruskal–Wallis test was used to test the effect of the number of clones on both the total number of sporocysts per snail and the mean number of sporocysts per clone (i.e. number of sporocysts/number of clones) as these variables did not meet the normality assumption of parametric tests. We also used a Pearson correlation test between the “parasitised” shell volume and the total volume of parasite within the single-clone infections to assess whether the modified shell shape is related to parasite growth.

Finally, the effect of the number of clones on shell shape was analysed using an ANCOVA with the number of clones as the main factor, total shell length as a covariate and width of whorl 1 as the dependent variable.

### 3. Results

#### 3.1. Shell shape and parasitism

Overall, 991 snails were measured and dissected, of which 170 (17.2%) were infected by the trematode *C. parvum*. *Coitocaecum parvum*-infected snails were not only significantly smaller in length (mean =  $2.221 \pm 0.034$  mm) than their uninfected counterparts (mean =  $2.466 \pm 0.025$  mm; ANOVA,  $F_{1,989} = 17.461$ ,  $P = 0.00003$ ), but they also displayed altered shell shape compared with uninfected individuals. The ANCOVA comparing the two snail classes indicated that individuals parasitised by *C. parvum* had significantly narrower shells: the ratio of width of whorl 1 to total length was smaller ( $F_{1,988} = 383.85$ ,  $P < 0.0001$ ; Fig. 2) within the infected snail class. Parasitism was related to the degree of spinosity as well: infected snails were less spiny than uninfected individuals ( $\chi^2 = 535.63$ ,  $df = 3$ ,  $P < 0.0001$ ; Fig. 3). Altogether, snails infected by the trematode *C. parvum* displayed significant alterations of shell morphology (size, shape and spinosity).

The effects of parasitism on total and “parasitised” shell volumes (Fig. 1) proved very interesting in terms of the space available for the parasite (Fig. 4). Indeed, whereas the total shell volume of infected snails is significantly smaller than that of their uninfected counterparts for a given length (ANCOVA,  $F_{1,259} = 553.17$ ,  $P < 0.0001$ ; Fig. 4a), the volume inside the shell actually accessible to the parasite is significantly larger for any given snail length (ANCOVA,  $F_{1,259} = 405.29$ ,  $P < 0.0001$ ; Fig. 4b) in *C. parvum*-infected individuals. The “parasitised” shell volume as a function of the total shell volume was also significantly larger in infected snails than in their uninfected conspecifics (ANCOVA,  $F_{1,259} = 999.41$ ,  $P < 0.0001$ ; Fig. 4c). Infected snails had a greater “parasitised” volume to total volume ratio as total volume increased (Fig. 4c).

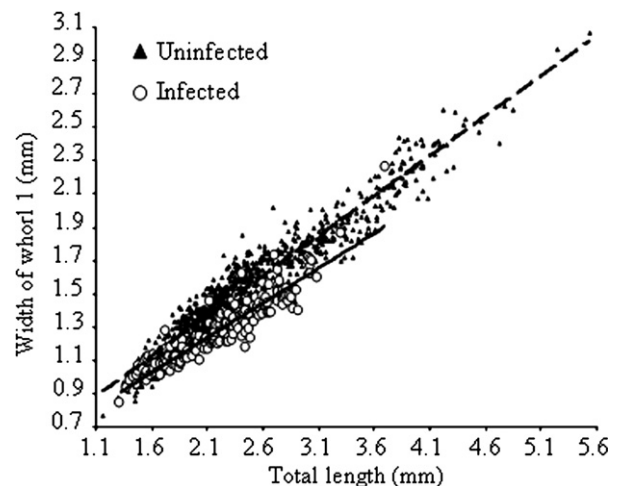


Fig. 2. Relationship between length and width of whorl 1 among uninfected ( $n = 821$ ) and *Coitocaecum parvum*-infected ( $n = 170$ ) snails. The lines of best fit are linear regressions.



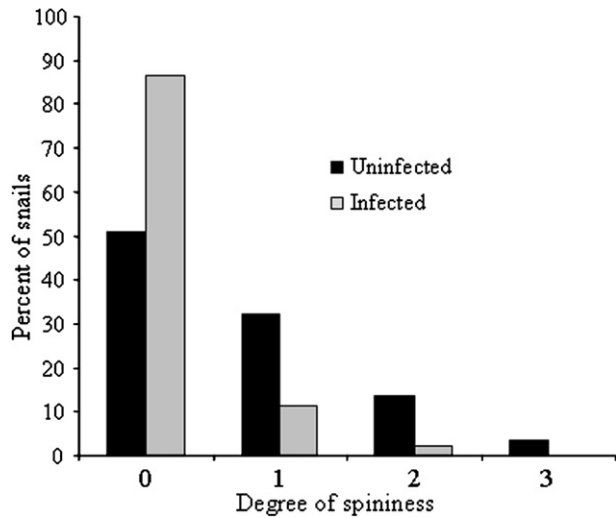


Fig. 3. Proportion of uninfected ( $n = 821$ ) and *Coitocaecum parvum*-infected ( $n = 170$ ) snails in each spinosity class (from 0 = smooth shell to 3 = fully developed spines).

Although *C. parvum* infections were associated with alterations in the shape and size of the snail *P. antipodarum*, there was no relationship between parasite infections and shell mass (i.e. thickness). The dry shell mass of uninfected snails (mean =  $0.664 \pm 0.02$  mg) was not significantly different from the shell mass of infected individuals (mean =  $0.62 \pm 0.02$  mg; ANCOVA,  $F_{1,97} = 0.759$ ,  $P = 0.39$ ).

### 3.2. Parasite clonal diversity, sporocyst volume and host shell morphology

The eight loci used for this study possessed three to 15 alleles and observed heterozygosities of 0.03 to 1.00 (Table 1). All loci deviated significantly from Hardy–Weinberg expectations ( $P < 0.0001$ ) and significant disequilibrium was detected between Cpa-26 and three other loci (Cpa-12, Cpa-19 and Cpa-28). The majority of observed deviations from Hardy–Weinberg expectations were not unexpected as a high proportion of parasites produce eggs by selfing (progenesis). This reproductive strategy can increase  $F_{IS}$  values (Jarne and Auld, 2006) as detected in our study (overall  $F_{IS} = 0.470$ ). Two loci (Cpa-3 and Cpa-4) displayed heterozygote excesses and may reflect the influences of processes such as clonal reproduction by miracidia (Balloux et al., 2003) (although this will affect the entire genome and would likely be exhibited by a majority of loci) or associative overdominance (Ohta and Kimura, 1970) on these loci.

Amongst the 46 snails used for this part of the study, 34 individuals possessed only one parasite genotype and 12 snails possessed two or more genotypes (Table 2). Overall, 66 parasite genotypes were found, of which 32 (48.5%) were recovered from mixed-clone infections. When several parasite clones were found inside the same host, one clone always dominated numerically (Table 2). Only the snail

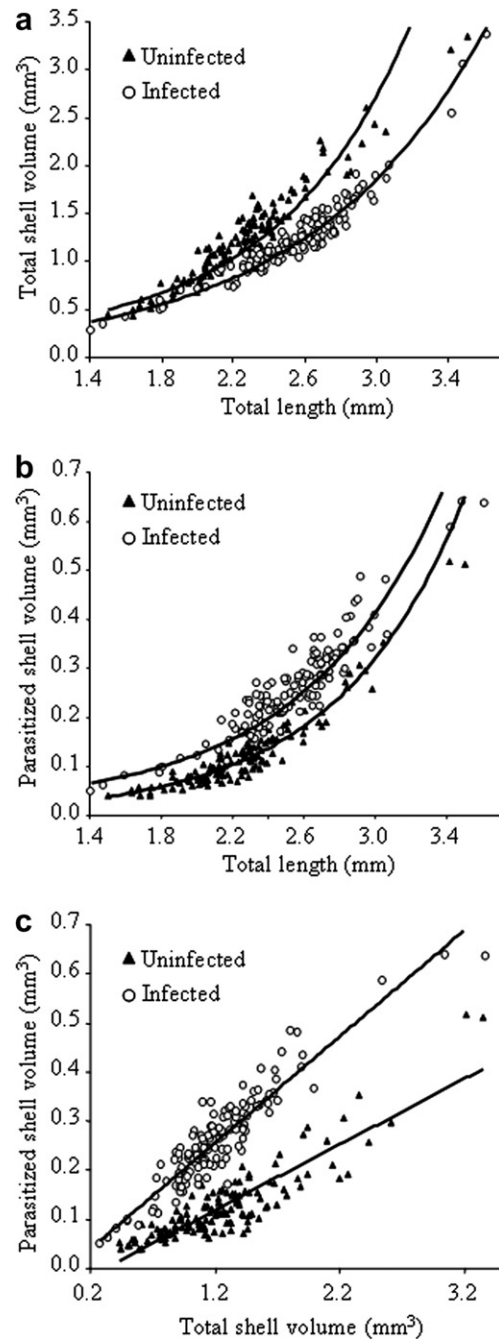


Fig. 4. Relationship between: (a) length and total shell volume, (b) length and parasitised shell volume and (c) total shell volume and parasitised shell volume among uninfected ( $n = 131$ ) and *Coitocaecum parvum*-infected ( $n = 131$ ) snails.

with five different clones showed equality between two pairs of non-dominant clones (nine sporocysts shared the same genotype, two pairs had the same genotype and the two remaining sporocysts possessed single genotypes) resulting in 60% of the sporocysts possessing the “dominant” clone, 26.6% the second clones and 13.4% the third clones in the hierarchy. Thus, the snails containing more than two parasite genotypes (i.e. five snails with three clones and one with five) were grouped together for subsequent analyses.

Table 1  
Number of alleles (*A*), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ) and  $F_{IS}$  for each microsatellite locus and across all loci used for the genotyping of *Coitocaecum parvum* sporocysts

Locus	<i>A</i>	$H_O$	$H_E$	$F_{IS}$
Cpa-3	3	1.000	0.565	−0.769
Cpa-4	3	0.848	0.577	−0.471
Cpa-8	15	0.108	0.881	0.878
Cpa-12	13	0.212	0.886	0.761
Cpa-19	14	0.182	0.866	0.790
Cpa-26	7	0.288	0.755	0.619
Cpa-28	3	0.106	0.426	0.751
Cpa-29	3	0.030	0.286	0.894
All loci	61	0.347	0.655	0.470

The total number of sporocysts per snail significantly increased with the number of parasite genotypes (Kruskal–Wallis ANOVA,  $H_{2,46} = 15.86$ ,  $P < 0.0001$ ; Fig. 5a). However, this increase was still below the theoretical increase expected if multiplication of each clone is independent from the presence of other clones (Fig. 5a). In fact, no difference was found in the total volume of parasite tissues contained in the shell between snails with one clone of *C. parvum*, snails with two clones and snails with three or more clones (ANOVA,  $F_{2,43} = 0.331$ ,  $P = 0.72$ ; Fig. 5a). It is consistent with the fact that, within the single clone infections, we found a significant correlation between the “parasitised” shell volume and the total parasite volume (Pearson  $r = 0.37$ ;  $P = 0.030$ ). This result suggests that the parasite uses most of the space available inside the shell. As a result, not only the number of sporocysts per clone declines with an increase in the number of parasite clones (Kruskal–Wallis ANOVA,  $H_{2,46} = 45$ ,  $P < 0.0001$ ; Fig. 5b) but also the mean sporocyst volume, and consequently the production of cercariae (Théron and Touassem, 1989), significantly decreases with an increasing number of clones (ANOVA,  $F_{2,43} = 4.88$ ,  $P = 0.012$ , Fig. 5b). Despite the apparent competition between parasite clones sharing the same host, at least for the space inside the snail, the alteration of the shell shape of infected individuals was not more pronounced in snails with multiple-clone infections (Fig. 6). There was no significant difference in shell shape between snails containing a single-clone and those containing two or more distinct clones of *C. parvum* (ANCOVA,  $F_{2,42} = 0.117$ ,  $P = 0.89$ ).

Table 2

Genotypes of sporocysts of *Coitocaecum parvum*, showing the number of snails with mixed-clone infections and the proportion (%) of sporocysts from each clone

Number of clones	Number of snails	Mean percentage of sporocysts from each clone				
		Clone 1	Clone 2	Clone 3	Clone 4	Clone 5
1	34	100 (416)	–	–	–	–
2	6	62.2 (74)	37.8 (45)	–	–	–
3	5	50.8 (64)	34.1 (43)	15.1 (19)	–	–
4	1	60 (9)	13.3 (2)	13.3 (2)	6.7 (1)	6.7 (1)

The actual numbers of sporocysts are indicated in parentheses after each percentage value. The mean number of sporocysts per snail was  $14.7 (\pm 0.9)$ .

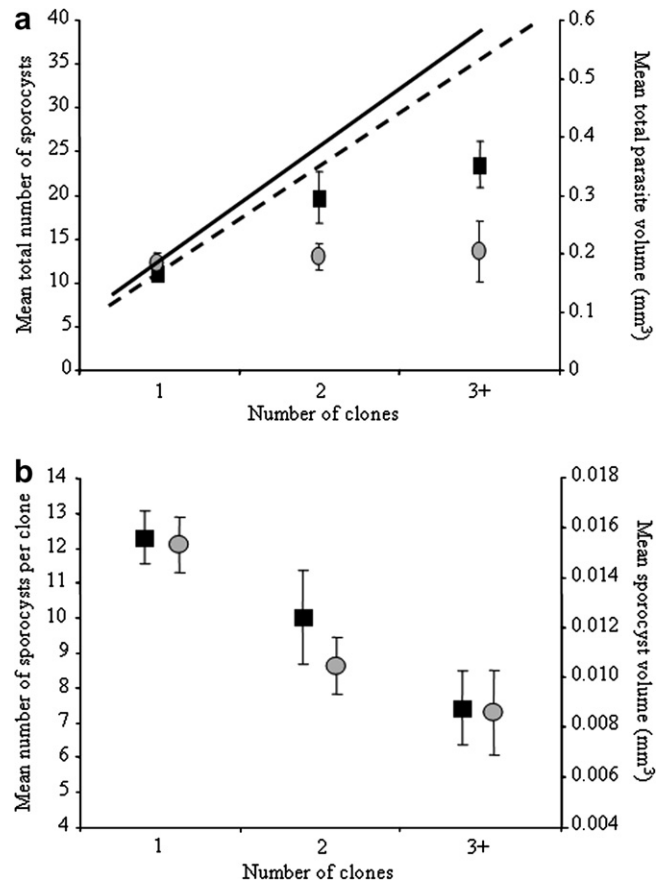


Fig. 5. Variations in: (a) mean ( $\pm$ SEM) total number of sporocysts ( $\circ$ ) and mean ( $\pm$ SEM) total parasite volume ( $\square$ ) as a function of the number of trematode clones in the snail. The plain line represents the theoretical increase in “volume” and the dotted line the theoretical increase in the “number of sporocysts” given by the values for single-clone infections multiplied by the number of clones. (b) Mean ( $\pm$ SEM) number of sporocysts per clone ( $\circ$ ) and mean ( $\pm$ SEM) sporocyst volume ( $\square$ ) as a function of the number of clones in the snail. Among the 46 snails dissected, 34 had one parasite clone, six had two and six had three or more.

#### 4. Discussion

Intra-host competition between conspecific parasites has been predicted to select for higher levels of host exploitation because resources within a host are limited and vital to each parasite genotype (Frank, 1992, 1996; May and Nowak, 1995). This may be particularly true for trematode

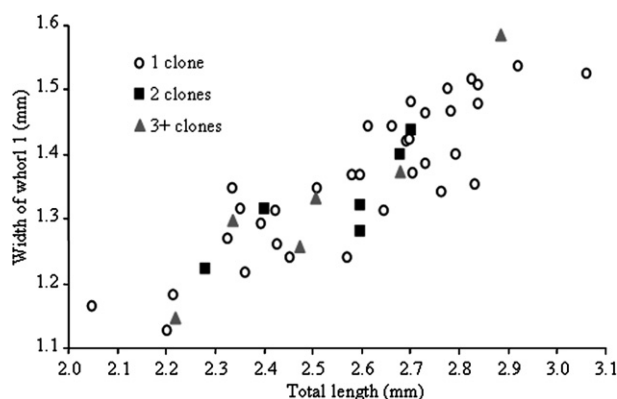


Fig. 6. Relationship between length and width of whorl 1 in snails infected by 1 ( $n = 34$ ), 2 ( $n = 6$ ) or  $\geq 3$  ( $n = 6$ ) different clones of the trematode *Coitocaecum parvum*.

parasites in their snail intermediate host. In this case, not only the energy that the parasite can divert from its host is limited but the space inside the snail shell available for parasite development is also physically restricted. The enhanced growth observed in trematode-infected snails is thought to be either a non-adaptive byproduct of host castration, a host counter adaptation to improve reproductive output, or an adaptation by the parasite to increase the space available in the shell (see Minchella et al., 1985 and references therein; McCarthy et al., 2004). Under any of these hypotheses, snails infected by several parasite clones could display more pronounced shell modifications than snails infected by only one trematode genotype. For the parasite adaptation hypothesis, the higher combined levels of host exploitation could induce a more profound alteration of the host phenotype. However, while the effects of trematode parasites on the shell morphology of their snail hosts have received much attention and are now well documented, only a few studies have empirically tested the effect of mixed-clone infections on the snail host and those studies focused exclusively on either host fecundity and survival (Davies et al., 2002) or parasite reproductive success (Gower and Webster, 2005). We believe our study is the first to test for both the overall effect of a trematode on the shape of its snail host and the possible additive effect of multi-clone parasite infections.

It is clear that infection with *C. parvum* is associated with alterations in snail shell shape, but not with simple uniform increases or decreases in size. Infected *P. antipodarum* are significantly less spiny. Whether the infection influences spine production or the genetic background of hosts is a factor (i.e. snails with smooth shells are more likely to be infected) is unclear (Levri et al., 2005). However, since spines are energetically costly to produce (Winterbourn, 1970), it is reasonable to assume that snails infected by trematodes cannot afford to produce any shell ornamentation when parasitism already consumes a significant amount of resources from the host. This result is consistent with observations made by Levri et al. (2005); they found that field-collected *P. antipodarum* parasitised by

*Microphallus* sp. were less likely to produce spines than their uninfected conspecifics. Thus, reduction in the production of protective morphological structures seems to be a general consequence of parasitism in natural populations of *P. antipodarum*. However, whether infected snails cannot sustain the production of spines because of the direct cost of infection or the parasite directly inhibits the production of ornamentation in its host and diverts this energy to its own use (development and/or reproduction) remains unclear.

Levri et al. (2005) found that field-collected *P. antipodarum* infected by *Microphallus* sp. were significantly longer and had a greater width-to-length ratio than uninfected individuals but could not determine whether these effects were specific to *Microphallus* or not. Therefore, they suggested that changes in shape and defenses (i.e. spines) observed in trematode-infected snails were a general response to parasitism. However, in our study using the same measurements, uninfected snails displayed the same width-to-length ratio as in the study by Levri et al. (2005) but *C. parvum*-infected individuals showed shell modifications opposite to those associated with *Microphallus* infection. *Coitocaecum parvum*-infected snails were smaller than uninfected individuals, and therefore smaller than *Microphallus*-infected snails, and had a smaller width-to-length ratio, suggesting that different trematode species may have specific effects on their snail hosts. Parasite-induced gigantism in snails infected by castrating trematodes (Minchella et al., 1985; Gorbushin, 1997) is thought to be either a non-adaptive side-effect of infection (Sorensen and Minchella, 2001), the energy disrupted from reproduction being reallocated to growth (Ballabeni, 1995), or an adaptive parasite manipulation of host physiology to increase host volume and/or survival and increase the parasite transmission rate (McCarthy et al., 2004). This might be true when, as in *Microphallus* sp., host survival is required for transmission to the next host. In our case, there is a trade-off between producing numerous cercariae in a short period of time, at the risk of increasing host mortality, and producing fewer cercariae but over a long period. We found that *P. antipodarum* parasitised by *C. parvum* had a significantly smaller overall volume than uninfected snails. Whether this result is due to a decreased growth rate or an increased mortality rate of infected snails is unclear but tends to indicate that *C. parvum* exerts a greater pathogenic effect in terms of host resources exploitation, than the trophically transmitted *Microphallus* sp. However, given the reduced overall size of infected snails, the parasite seems to be making the best of a bad situation by specifically increasing the volume of the shell section available for its development (i.e. the part of the shell originally containing the gonads). It would be interesting to examine whether the differences in host phenotype alteration detected between *Microphallus* and *Coitocaecum* are associated with differences in the timing of infections during the host's life cycle (see Sorensen and Minchella, 2001), but we cannot address this with our data.

When parasite genotypes exist frequently in mixed-genotype infections in the population and there is within-host competition for resources, increased levels of host exploitation and/or virulence may be a viable solution for the parasite. In our study, almost half of the parasite genotypes were found in multiple-clone infections, the parasite utilises most of the available space inside the snail shell and, on average, each clone in a mixed-infection produces fewer and smaller sporocysts than clones occurring alone inside a snail. We also observed that mixed-clone infections are numerically dominated by one clone's sporocysts. Seemingly, there may be a conflict between clones when different genotypes infect the same snail; each clone might not have the same possibility to effectively achieve equal development (in terms of volume and number of sporocysts) as a clone in single infection. This can mean that sharing a host with other clones leads to a reduction in the rate at which a clone multiplies to produce cercariae. This fitness cost could be mitigated by further alterations of snail morphology to further increase the space available for the parasites. However, we found no significant effect of mixed-infections on snail shape. The shells of snails harbouring multiple-clone infections were not significantly different from those of snails with only one parasite genotype. Thus, snails harbouring two or more clones of *C. parvum* do not display greater modifications of shell morphology; whether or not other features of the host morphology or physiology are affected cannot be determined from this study. These results suggest that, even in single-clone infections, the trematode parasite *C. parvum* has a substantial impact on its first intermediate host that may be close to the maximum tolerated by the snail. This is consistent with the fact that if mixed infections are frequent, a high level of host exploitation can become fixed in the parasite population. If shell changes are the result of host responses rather than parasite modifications, our data indicate that hosts respond equally to single and multi-clone infections. There exists an alternative explanation for our observations that multi-clone infections are dominated by one clone and that alterations of shell shape do not differ between single-clone and multi-clone infections: there could be a significant time gap between the first infection and subsequent infections. In this scenario, the first clone to infect a snail induces a change in shell growth and occupies most of the space available, such that clones arriving later have little room to occupy and cannot alter the host growth patterns already determined by the first clone. Two basic facts, however, suggest that the time gap between infections must generally be small: snails seem to mostly acquire infections early in life, when they are still sexually immature (Zakikhani and Rau, 1999), and the prevalence of infection is much higher in the smaller sized snails. These findings suggest that snails are exposed to many clones for only a short period of time, with no further infections later in life. Thus, the explanations we provide earlier are plausible although not exclusive.

In conclusion, parasitism by *C. parvum* in *P. antipodarum* is related to shell shape (and therefore shell volume) and defense ornamentation in a way that the parasite may modify its host morphology to maximise resource exploitation by diverting resources normally allocated to production of shell ornamentation. Infected snails display a lower degree of spinosity and tend to be smaller. The shell shape is also modified and tends to be narrower for a given length, decreasing the overall volume of the shell. However, the part of the shell potentially available for the parasite is significantly larger in infected individuals. The relatively high proportion of multiple-clone infections may have selected for an increased level of host exploitation in the parasite population. Altogether, our study suggests that changes in host morphology in trematode–snail associations could be parasite-specific compared with the study by Levri et al. (2005) and may depend on the frequency of multiple-clone infections within the population.

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