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Trematode-induced alterations in shell shape of the mud snail *Zeacumantus subcarinatus* (Prosobranchia: Batillariidae)

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The shell morphology of mud snails, *Zeacumantus subcarinatus*, both uninfected and infected by trematodes, was compared to determine if different trematode species induce different degrees of alteration in host shell shape. Snails harbouring either the echinostomatid *Acanthoparyphium* sp., or a double infection by the microphallid *Maritrema novaezealandensis* and an undescribed philophthalmid species, had a wider shell base relative to the rest of the shell spire, than uninfected snails or snails infected by only one of the latter two trematode species. These results are independent of any differences in shell length among the different infection groups. The findings of this study suggest that alterations in host shell morphology are species-specific trematode manipulations of host phenotype rather than a generalized host response to castrating trematodes.

INTRODUCTION

Trematodes are the most common parasites infecting gastropods worldwide (Poulin & Mouritsen, 2003). In most cases, trematode infections cause changes in either the survival, growth, reproduction or behaviour of their snail hosts (e.g. Sousa, 1983; Curtis, 1987; Huxham et al., 1993; Gorbushin, 1997; Gorbushin & Levakin, 1999; Probst & Kube, 1999). The partial or complete host castration induced by trematodes in snails is often followed by the allocation of more resources to growth, with the result that infected snails can grow to larger sizes than their uninfected conspecifics (Mouritsen & Jensen, 1994; Gorbushin, 1997; Probst & Kube, 1999). The adaptive nature of this phenomenon, known as gigantism, has been debated for years (Sousa, 1983; Minchella, 1985; Gorbushin & Levakin, 1999; Sorensen & Minchella, 2001). It is still unclear whether increased growth in parasitized snails is an adaptive host response that could, for instance, serve the snail to live longer and outlast the infection, or an adaptive manipulation of the host's phenotype by the parasite that could increase its transmission success.

Trematodes are known to influence not only the growth rates of their snail hosts, but also their shape (Krist, 2000; McCarthy et al., 2004). For instance, infection by the trematode *Microphallus piriformes* caused periwinkles *Littorina saxatilis* to grow relatively longer and narrower shells than uninfected conspecifics (McCarthy et al., 2004). If altered shell shape as well as gigantism are general responses of snails to castration and relatively greater allocation of resources to growth, then we might expect all castrating trematodes to induce similar changes in shell shape. In contrast, if these alterations in shell shape are specific adaptations of the parasite, we would expect different parasite species to differ in their ability to influence shell morphology. Thus, in systems where one snail population harbours several trematode species, it should be possible to use differences in shell shape associated with different parasites as a more sophisticated way of determining whether these are general host responses or specific parasite manipulations.

Here, we use the mud snail, *Zeacumantus subcarinatus* Sowerby, 1955 (Prosobranchia: Batillariidae) for such an investigation. This snail, highly abundant in New Zealand soft-sediment intertidal communities, serves as first intermediate host to several trematode species, three of which are relatively common. First, the microphallid *Maritrema novaezealandensis* multiplies within the snail before its cercariae leave the snail to encyst in crustaceans (Martorelli et al., 2004). This species is consistently the most prevalent of the trematodes in *Z. subcarinatus* at our study site. Second, the echinostomatid *Acanthoparyphium* sp. also uses the snail as first intermediate host; its cercariae then go on to encyst in bivalves. Third, an undescribed philophthalmid species commonly occurs in the snails, from which cercariae emerge to encyst on hard surfaces, such as mollusc shells or crustacean exoskeletons. All three species complete their life cycle when they are ingested by shore birds along with their molluscan or crustacean second intermediate hosts. All three trematodes also castrate the snail host; in fact, because of their relatively high combined prevalence in some areas, they can have measurable impacts on the population density and biomass of the snail *Z. subcarinatus* (Fredensborg et al., 2005).

In the present study, we compared the shell size and shell shape of snails infected by each of the three trematode species. By focusing on differences in the effects of different parasite species on the same host species, our study sheds some light on whether changes in snail growth following infection are host or parasite adaptations.

MATERIALS AND METHODS

Approximately 200 *Zeacumantus subcarinatus* snails were collected from Lower Portobello Bay, Otago Peninsula, in

June 2004. Snails were collected from under small rocks in the upper intertidal zone. In the laboratory, each snail was placed in an individual Petri dish, half filled with seawater. The dishes containing the snails were incubated at a temperature of 25°C for 14 h. This procedure induces cercarial production and release by infected snails, and facilitates the identification of infected snails. After the incubation period, each Petri dish was examined under a dissecting microscope. The presence or absence of cercariae in each dish, and their identity, were recorded. The incubation and examination of dishes for cercarial release was repeated twice, the first time one week and the second time one month after snails were collected in the field. This was performed to confirm the infection status of the snails, and reduce the risk of misclassification of infected or uninfected snails.

Based on cercarial release, five groups of snails were established: group U, uninfected snails, N=55; group M, snails infected by *M. novaezealandensis*, N=60; group P, snails infected by the philophthalmid, N=33; group A, snails infected by *Acanthoparyphium* sp., N=22; and group D, snails harbouring a double infection of both *M. novaezealandensis* and the philophthalmid species, N=10. Snails harbouring rare trematode species, or other combinations of the three common species, were excluded from the analysis due to small sample sizes.

Each snail was measured individually. All shell measurements were taken using Vernier callipers (±0.02 mm). The maximum length of each shell was measured from the base of the aperture to the tip of the shell's apex. The maximum width of each snail was measured from the widest point across the base of the shell. Preliminary observations suggested that the different groups of snails varied with respect to the size of the bulge at the base of the shell relative to the diameter of the rest of the shell spire. Thus, the ratio between the width of the third whorl of the shell (third from the aperture) and the maximum width was chosen as a measure to compare shell shape across the five infection groups. The maximum width of the third whorl was divided by the maximum basal width of the shell to give the value hereafter referred to as the width ratio: a small ratio represents a shell very wide at the base relative to the spire, and large ratios indicate shells with a relatively narrow base.

The snails were later dissected to confirm their infection status. Because they were used in other studies following the measurements and were not individually marked, the few misclassified snails could not be deleted from the data set. Overall, 83% were classified correctly. Errors occurred either in the *M. novaezealandensis* group, where some snails harboured immature infections of a second trematode species, or in the uninfected group, where some snails were in fact in the early stages of infection. Because the undetected infections were clearly recent and unlikely to have affected shell growth, and because the two groups of snails in which classification errors occurred were not the ones with unusual shapes (see Results), these errors have no bearing on our findings.

We verified the normality and homoscedasticity of the data. Only shell length data did not meet these assumptions, and had to be $\log_{10}$-transformed. All analyses were performed using standard parametric tests.

RESULTS

There was a significant difference in the width ratio of shells among the different infection groups (one-way analysis of variance, $F_{4,175}=5.39$, $P<0.001$). Based on Tukey's pairwise comparisons among means, both the double infection group and the *Acanthoparyphium* sp. group have significantly lower ratios than the other three groups (all $P<0.05$), but did not differ from each other ($P=0.21$) (Figure 1). This indicates that these two groups have a larger bulge at the base of the shell, in relation to the rest of the shell spire, than the other groups.

The maximum shell length also differed significantly between the different infection groups ($F_{4,175}=12.04$, $P<0.001$). The *Maritrema novaezealandensis* and uninfected groups had significantly shorter shell lengths compared with the other three groups (Figure 1). Across all 180 snails from all five groups pooled, there was a significant negative relationship between the width ratio and the log-transformed maximum shell length (linear regression: $F_{1,178}=16.34$, $r^2=0.08$, $P<0.001$). Thus the width ratio decreases as shell length increases (regression equation: $y=1.06-0.0335x$).

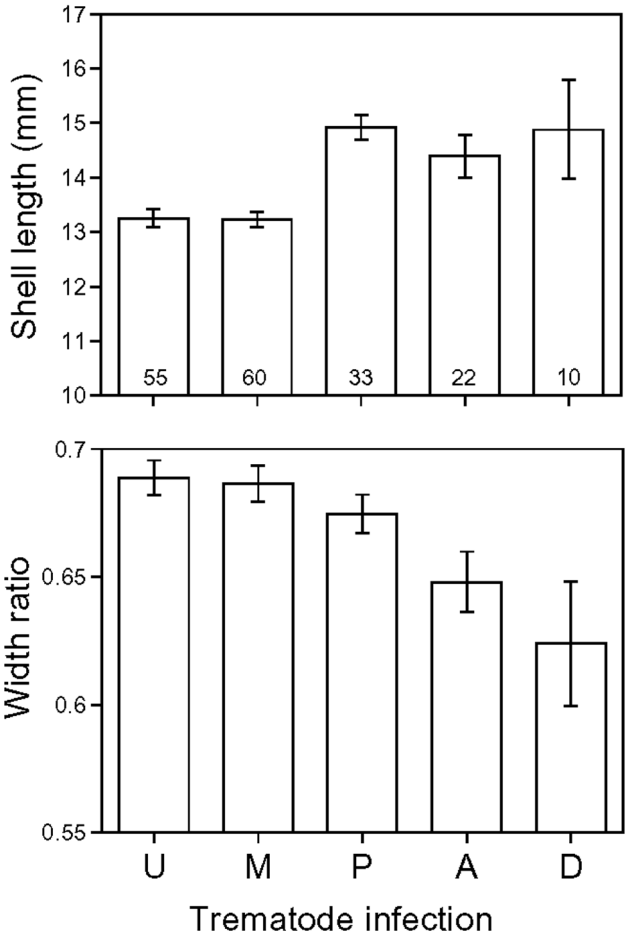

Figure 1. Mean (±SE) maximum shell length (top) and ratio between the width of the third whorl and that of the shell base (bottom), for the different infection groups of the snail *Zeacumantus subcarinatus*. The groups are: U, uninfected; M, infected by *Maritrema novaezealandensis*; P, infected by a philophthalmid species; A, infected by *Acanthoparyphium* sp.; and D, infected by both *M. novaezealandensis* and the philophthalmid species. Numbers at the base of the bars represent sample sizes.

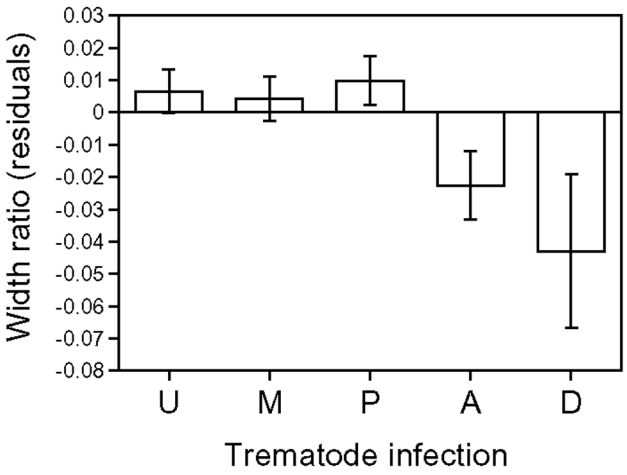

Figure 2. Mean (±SE) ratio between the width of the third whorl and that of the shell base, corrected for shell length, for the different infection groups of the snail *Zeacumantus subcarinatus*. The data are residuals from the regression between width ratio and maximum shell length. The groups are: U, uninfected; M, infected by *Maritrema novaezealandensis*; P, infected by a philophthalmid species; A, infected by *Acanthoparyphium* sp.; and D, infected by both *M. novaezealandensis* and the philophthalmid species. Sample sizes are as in Figure 1.

To account for the fact that width ratio is not independent of shell length, we used the residuals from the above regression as measures of the width ratio corrected for shell length. A comparison of these residuals showed a significant difference among the snail groups in width ratio corrected for shell length ($F_{4,175}=3.44$, $P=0.010$). The double infection group and the *Acanthoparyphium* sp. group both had lower width ratios than would be expected for any given shell length, lower also than those of the other three snail groups (post-hoc Tukey tests, all $P<0.05$), which had slightly higher ratios than expected from their shell length (Figure 2). This size-corrected result confirms the significance of the original analysis on the raw data, and clearly shows that the double infection and *Acanthoparyphium* sp. groups have a wider bulge at the base of the shell in comparison with the rest of the shell spire than any of the other groups.

DISCUSSION

Altered growth rates are well-documented consequences of trematode infection in snail intermediate hosts (Sousa, 1983; Minchella, 1985; Mouritsen & Jensen, 1994; Gorbushin, 1997; Gorbushin & Levakin, 1999; Probst & Kube, 1999; Sorensen & Minchella, 2001). Changes in shell shape, independent of growth or size of snails, are also observed following trematode infection (Krist, 2000; McCarthy et al., 2004). Our study allowed us to determine whether such changes in shell shape are generalized responses of snails to trematode-induced castration, or whether they are specific to different species of trematodes. We found that the snail *Zeacumantus subcarinatus*, when harbouring either the trematode *Acanthoparyphium* sp., or double infections by *Maritrema novaezealandensis* and the philophthalmid species, displayed a wider shell base relative to the shell spire than individuals infected by only one of the latter two species or uninfected snails. This suggests that altered shell shape is not a general life-history response in castrated snails. Instead, different species of trematodes can induce different degrees of changes in shell shape in their common host, whether these changes are adaptive for the parasites or merely by-products of infection.

The parasite may derive benefits from alterations in host shell shape. McCarthy et al. (2004) found that the changed shell shape of the periwinkle *Littorina saxatilis* caused by the trematode *Microphallus piriformes* resulted in a 12% increase in the total volume inside the shell. *Microphallus piriformes* has an abbreviated life cycle in which the cercariae do not leave the snail host, but instead grow directly into metacercariae and remain within the snail. A greater volume within the host shell would provide more space for the accumulation of greater numbers of metacercariae. The trematodes in our study all produce cercariae that leave the snail to encyst elsewhere. Still, there is competition for space within the shell between host and parasite tissues (Gerard & Théron, 1995). The bulge at the base of the shell in *Z. subcarinatus* harbouring *Acanthoparyphium* sp. or double infections indicates that the diameter of the more recent whorls has increased, with a resulting increase in volume within the shell. However, because larval trematodes in snails are associated with the gonad–digestive gland complex, positioned in the uppermost part of the shell spire, it is unclear how a small increase in the volume of the shell base would benefit the parasites. Nevertheless, this growth alteration may allow the parasites within the shell to occupy more space and achieve enhanced cercarial production.

This raises an obvious question: why do all three trematode species not induce this alteration in growth to the same degree? It seems from our results that *Maritrema novaezealandensis* and the philophthalmid species must combine their effects and act synergistically, when they occur in the same snail, to match the alterations that *Acanthoparyphium* sp. can induce on its own. Based on the review of published results carried out by Sorensen & Minchella (2001), trematode species with rediae were somewhat more likely to induce gigantism in their snail host than those using sporocysts only. Within the snail host, rediae and sporocysts are different types of developmental stages; the former cause more physical damage to host tissues than the latter, because they can feed directly on the host (Kearn, 1998). In our study system, both the echinostomatid *Acanthoparyphium* sp. and the philophthalmid species have rediae, whereas the microphallid *M. novaezealandensis* has only sporocysts. Therefore, the explanation for the differences in shell shape alterations induced by these parasites must lie elsewhere. Possibly there exist differences between the three trematode species with respect to their rates of production of cercarial biomass, and these may relate to the degree of shell shape modification they induce. Whatever the explanation, our results have shown that different species of trematodes, despite all castrating their host, induce different changes in shell morphology in their common host, the mud snail *Zeacumantus subcarinatus*. This result points toward changes in shell growth being either an adaptive, species-specific parasite manipulation of host phenotype, or a species-specific by-product of infection.

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