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Williams, A and Rae, R (2016) Cepaea nemoralis (Linnaeus, 1758) uses its shell as a defence mechanism to trap and kill parasitic nematodes. Journal of Molluscan Studies, 1-2. ISSN 0260-1230

RESEARCH NOTE

CEPAEA HORTENSIS USES ITS SHELL AS A DEFENSE MECHANISM TO TRAP AND KILL PARASITIC NEMATODES

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Slugs and snails are parasitised by a range of organisms including nematodes, bacteria, microsporidia, mites and flies (Barker, 2004). Of these, the nematodes are the most numerous and diverse as 108 species have evolved to parasitise molluscs (Grewal et al., 2003). One of these nematodes (*Phasmarhabditis hermaphrodita*) is a lethal parasite of a range of pestiferous slugs and snails including *Deroceras reticulatum*, *Arion ater* and *Helix aspersa* (Wilson *et al*., 1993). *P. hermaphrodita* has been formulated into a biocontrol agent (Nemaslug®) by Becker Underwood-BASF available for farmers and gardeners (Rae *et al*., 2007). Nematodes are mixed with water and applied using spraying equipment to soil where then go and search for potential gastropod hosts. They are attracted to slug mucus and faeces (Rae *et al*., 2006, 2009) and upon discovery they penetrate through the slugs' mantle and kill it between 4 and 21 days (Wilson *et al*., 1993; Tan & Grewal, 2001).

Unlike slugs, many snail species are resistant to *P. hermaphrodita* including *Oxychilus helveticus*, *Discus rotundatus, Achatina fulica* and *Clausilia bidentata* (Wilson *et al*., 2000; Coupland, 1995; Williams & Rae, 2015). It is unknown how these snail species can tolerate nematode infection, but recently it was shown that *A. fulica* could trap, encase and kill invading *P. hermaphrodita* in its shell. It is unknown if this process is evolutionary conserved and present in other snail species and to what extent it is affected by different colours of shell or even banding patterns. To answer this question we concentrated on using snails from the genus *Cepaea* which exhibit a range of different colour morphs including yellow, brown, pink, orange and red with up to five black longitudinal bands (Cain & Sheppard, 1954). The cause of differences in shell colour and banding has been discussed for decades and numerous factors have been suggested to be the reason including climate, predators, temperature and habitat change (Silvertown *et al*., 2011), but perhaps nematode parasites may play a role? There are conflicting reports on the susceptibility of snails of *C. hortensis* to *P. hermaphrodita*. Wilson *et al*. (2000) found that *C. hortensis* was susceptible but Rae *et al*. (2009) found *C. hortensis* was resistant. As no attention was paid to the differences in colour morph, maybe this difference in susceptibility was due to differences in colour morphs? Hence, we decided to repeat these experiments to understand if susceptibility towards *P. hermaphrodita* would differ due to specific colour and banding morphs of *C. hortensis* and to examine whether the this species of snail could trap *P. hermaphrodita* in their shells and whether this ability would alter with different colour and banding patterns.

C. hortensis were collected from Festival gardens, Liverpool and were stored in non-airtight boxes and fed *ad libitum* with cabbage and cucumber. *C. hortensis* were split into groups of either pink or yellow and then further split into 0 bands, 1 band and 3-5 bands. *P. hermaphrodita* were supplied by Becker Underwood-BASF, UK and stored at 10°C until use.

All six *C. hortensis* morphs were exposed to *P. hermaphrodita* at a rate of 30 nematodes per cm^2, which is the recommended rate of nematodes applied in the field (Wilson *et al*., 1993). Plastic non-airtight boxes (10 x 10 cm) were fitted with copper tape (to prevent snails from staying on the lid of box) and half filled with moist soil (approx. 25 g). Eighteen boxes had nematodes applied and eighteen had only water and no nematodes applied and acted as the control. Snails were fed with cucumber every 3-4 days. Survival was monitored every 3-4 days for 72 days. Survival of *C. hortensis* was compared using log rank test in OASIS (Yang *et al*., 2011). Numbers of nematodes encapsulated in the shells of different morphs of snails were compared using a one-way ANOVA.

P. hermaphrodita had no effect on the survival of yellow or pink *C. hortensis* with 0 bands, 1 band or 3-5 bands after 72 days exposure ($P>0.05$) (Table 1). At the end of the experiment, snails were dissected and the numbers of nematodes encased and killed in the shell were quantified. We found that *P. hermaphrodita* were trapped and killed in the shell of each morph but there were no significant difference between the numbers of nematodes encapsulated between the different morphs (P>0.05) (Table 1; Fig 1 a,b). Therefore, *C. hortensis* has the ability to trap and encase invading *P. hermaphrodita* but does not differ with banding pattern or colour.

C. hortensis is able to defend itself from *P. hermaphrodita* by producing shell tissue that seems to trap and encase invading nematodes. The nematodes appear as if perfectly preserved in amber and are completely covered by unknown cells. This ability is not affected by colour of the shell nor is it affected by the number bands the shell has. It remains to be seen how long it takes for the nematode to degrade or if it is preserved indefinitely in the shell. Interestingly, encapsulation of nematodes has also been shown in slugs. Rae *et al*. (2008) showed that *P. hermaphrodita* were trapped in large amounts in the shell of *Limax pseudoflavus* underneath the mantle. A characteristic sign of *P. hermaphrodita* infection of slugs is a swollen mantle (Wilson et al., 1993) due to the shell adding more calcareous tissue upon nematode contact. As this response is present in slugs and snails perhaps the gastropod shell is an ancient evolutionary conserved immune defense mechanism that is used to capture and kill invading parasites such as nematodes. It remains to be seen if the shell is used to protect snails from other invading parasites such as bacteria, microsporidia, mites and flies (Barker, 2004). Also as these nematodes are effectively preserved in the shell research could concentrate on understanding how prevalent infection of nematodes is in museum collections from around the world as all is needed is access to a shell collection and light microscope. It also remains to be see what cells are involved in this immune mechanism to recognise and trap these nematodes.

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TABLE TITLE

Table 1: Survival of different colour and banding morphs of *C. hortensis* exposed to *P. hermaphrodita* for 72 days and mean number of nematodes found encased in their shells.

Treatment	Colour	Number of bands	Mean percentage alive ± S.E.	Mean number of nematodes found in shell (range)
Control (no nematodes)	Yellow	0	86.67 ± 6.7	0
		1	100 ± 0	0
		5	93.33 ± 6.7	0
Control (no nematodes)	Pink	0	100 ± 0	0
		1	100 ± 0	0
		5	100 ± 0	0
Nematodes	Yellow	0	100 ± 0	7.38 (0 - 19)
		1	100 ± 0	8.63 (2 - 31)
		5	93.33 ± 6.7	12.88 (4 - 21)
Nematodes	Pink	0	100 ± 0	6.5 ± 1.97 (0 - 16)
		1	86.67 ± 13.3	14.5 ± 2.51 (2 - 23)
		5	93.33 ± 6.7	15.13 (3 - 28)

FIGURE LEGENDS

Fig 1: Numerous *P. hermaphrodita* encased and kill in the shell of a yellow *C. hortensis* (A) and close up of individual *P. hermaphrodita* trapped in pink *C. hortensis* (B). Scale bars represent 1mm (A) and 100 µm (B).