Do *Leucochloridium* sporocysts manipulate the behaviour of their snail hosts?

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Abstract

The conspicuous broodsacs of *Leucochloridium* spp. sporocysts, invading tentacles of their intermediate terrestrial snail hosts, are presented as a classic textbook example of the manipulation of host behaviour by a parasite. However, the conspicuous features indicated as facilitating the transmission of the parasite to its final avian hosts are characteristics of the appearance and behaviour of the parasite and not of its intermediate hosts. The demonstration that the sporocysts also manipulate the behaviour of the snails is still largely missing. In order to find out whether *Leucochloridium paradoxum* could manipulate the behaviour of its *Succinea putris* hosts, we compared the behaviour of *Leucochloridium*-infected snails with that of control animals (showing no signs of infection) living side by side, in the same habitat patches, in the field (Białowieża National Park, Poland). We had assumed that the 'moving caterpillar' display of the broodsacs was addressed to day-active, visually hunting, insectivorous birds and that the 'signalling' parasites should change the behaviour of their hosts to make the broodsacs more visible and/or more accessible to the group of predators mentioned. The infected snails with pulsating broodsacs behaved differently from their apparently non-infected counterparts. They moved farther, positioned themselves in more exposed and better illuminated places, situated higher in the vegetation. These alterations of behaviour would be beneficial for the parasites, would increase their visibility (detectability) and accessibility to the potential definite hosts. Thus, we demonstrated that, apart from their own phenotypic modifications, *L. paradoxum* sporocysts also changed the behaviour of their intermediate *S. putris* hosts. Such combination of modified host behaviour and strikingly visible parasite behaviour is rather unique, it is likely increasing the likelihood of parasite transmission to avian hosts.

Introduction

It is now widely recognized as true that an animal with a parasite is not likely to behave like a similar animal without that parasite (Moore, 2013). Such changed behavioural patterns could be beneficial for the parasite, for example, enhancing its transmission. Yet, in other cases, they could be just by-products of infection or they could benefit the host, that is, be part of its antiparasite tactics (see Moore, 2002 for review). When the parasite-induced behavioural changes benefit the parasite, they are described as 'manipulative'. There are several usages of this term (reviewed by Poulin, 2010), but it can be broadly defined as 'any alteration in behaviour that has fitness benefits for the parasite, such that the infected hosts behave in ways that facilitate the transmission or dispersal of the parasite, and therefore the completion of its life cycle' (Poulin, 2010). The idea of parasites taking control of host behaviour has attracted enormous attention of biologists

(e.g. Holmes & Bethel, 1972; Dawkins, 1982; Moore, 2002; Thomas, Adamo & Moore, 2005; Poulin, 1994, 2007, 2010); several hundred instances of host manipulation by parasites, spanning all major parasite groups, have been described (see review in Moore, 2002). Apart from simply documenting behavioural changes correlated with the parasites' presence, a growing number of experimental field studies have demonstrated that the parasite manipulations genuinely enhance parasite transmission (reviews in Moore, 2002; Poulin, 2007) and the knowledge of proximate neural mechanisms of the parasites' manipulation of hosts' behaviour has been rapidly increasing, as well (see e.g. special issue on 'neural parasitology', Adamo & Webster, 2013).

The conspicuous broodsacs of *Leucochloridium* spp. sporocysts invading tentacles of their intermediate terrestrial snail (usually *Succinea*) hosts, despite some cautionary notes (Moore, 2002; Casey et al., 2003), have become a classic textbook example (e.g. references above) of manipulation of host

behaviour by a parasite. The behaviour of *Leucochloridium* has also captured attention of the general public – see, for example, numerous video clips on the web showing 'zombi snails' manipulated by their 'mind-controlling' parasites. What is the evidence that *Leucochloridium* sporocysts manipulate the behaviour of their snail hosts? Unfortunately, it does not seem very strong. The conspicuous features that are indicated as facilitating transmission of the parasite to its final avian hosts are characteristics of the appearance and behaviour of the parasite and not of its snail hosts. When ready for transmission, the sporocysts form elongated extensions – broodsacs – that penetrate into the snail's protruding eyestalks during day time (Halík, 1931; Wesenberg-Lund, 1931). When in the tentacles, the contrastingly coloured, white, green/yellow and black-striped broodsacs, continuously pulsate at a rate of 60-80 contractions per minute (Halík, 1931; Wesenberg-Lund, 1931). Even after a tentacle has been ruptured, the projecting/separated broodsac continue to pulsate for a considerable time (Zeller, 1874; Wesenberg-Lund, 1931). The vividly pulsating broodsacs appear to mimic a crawling insect larva, which could increase the probability of their being preyed upon by birds, as suggested long ago (von Siebold, 1853). In a series of experiments on captive birds, Zeller (1874) showed that the pulsating broodsacs were attractive to potential definite hosts – insectivorous birds. The birds readily attacked the broodsacs, tore them out of tentacles and swallowed, sometimes only after striking them against a perch, as they would do with the real caterpillars. It seems, though, that these observations have never been confirmed in the wild (Moore, 2002). Actually, the only indication of possible manipulative changes of the snail behaviour comes from Mönning (1922, cited in Wesenberg-Lund, 1931) and Wesenberg-Lund (1931), who suggested that *Leucochloridium perturbatum*-infected snails sought well-lit and exposed places, on the upper sides of leaves, which would make the contracting broodsacs more accessible and visible to avian predators. To best of our knowledge, this assertion has never been tested.

To learn whether *Leucochloridium* manipulates the behaviour of its *Succinea* hosts, we compared the behaviour of *Leucochloridium*-infected snails and of control (showing no signs of infection) animals living side by side, in the same habitat patches, in the field. Following suggestions of earlier authors (see above), we had assumed that the 'moving caterpillar' display of the broodsacs was addressed to day-active, visually hunting, insectivorous birds. Thus, the 'signalling' snails should change the behaviour of their hosts to make the broodsacs more visible and/or more accessible to the group of predators mentioned. They could achieve this in several different ways (review in Moore, 2002): by making their hosts more mobile, remaining active for longer periods, staying in more open and better illuminated places or higher up in the vegetation. We checked if the behaviour of the infected snails differed from the control ones in the predicted fashion.

Study area and methods

We carried out observations in the Białowieża National Park (Poland), known to have parasitized snails (Pojmańska, 1958).

We chose a riverine forest patch (compartment 398, plot K, Wesołowski *et al.*, 2010), where we regularly observed *Leucochloridium*-infected snails (own unpublished data). It was alluvial, open canopy forest, composed mostly of *Alnus glutinosa* with an admixture of *Fraxinus excelsior* and *Picea abies*. The ground vegetation was very lush, consisting mostly of *Urtica dioica*, *Phragmites australis*, *Cirsium oleraceum*, *Carduus personata*, *Iris pseudoacrous*, *Caltha palustris*, *Filipendula ulmaria*, *Scirpus sylvaticus*. The plants formed an almost continuous dense ground cover, the tallest stems exceeding 1.5 m. During the study period, the ground remained wet, at places covered with surface water.

We slowly moved through the area, looking for snails with pulsating broodsacs (= 'infected' further on). To avoid repeated observations of the same individuals, each time, we searched for them in different parts of the study area. To minimize the impact of possible confounding variables (time of the day, temperature, cloudiness, microhabitat), we attempted to simultaneously observe the behaviour of the 'infected' and of the 'non-infected' snails. Therefore, after spotting an 'infected' individual, we scrutinized the vegetation in its close neighbourhood, down to the ground level, to locate 'non-infected' snails, that is, individuals of similar size, but showing no signs of infection (extended bases of tentacles, Wesenberg-Lund, 1931). However, as these could include *Leucochloridium*-infected snails, but with sporocysts not forming broodsacs yet (impossible to detect in the field, Wesenberg-Lund, 1931), herein we use a more neutral 'control' term to describe the reference snails. After finding in pilot observations (not included) that we were able to observe and record the behaviour of no more than four snails at the same time, we matched each infected snail with three control ones. Before starting the behavioural observations, we recorded the date and time of day, identified the snail species (following the key by Wiktor, 2004) and species of the parasite (using colouration patterns of broodsacs Pojmańska, 1969; Casey *et al.*, 2003; Zhukova *et al.*, 2012).

We observed snails from some distance so as not to touch plants on which they were staying and not to cast shade on them. Each observation session lasted 45 min. We were observing the behaviour of snails continuously, but recorded it every 15 min, which yielded four observations per individual. At each instant, we recorded the following variables:

• The height above the ground, measured to the nearest 5 cm with a pocket tape measure.

• Illumination (to the nearest 5 lux): We used a Konica Minolta T-10 M meter with a mini receptor head and measuring range up to 299 000 lux. The receptor head was connected by a flexible cable to the main device's body. We placed the receptor next to a snail (without touching it) with the receptor window facing upwards in order to measure the amount of down welling illumination. We took the measurements in the NORMAL FAST mode of the light meter.

• Activity: 0 = inactive (tentacles hidden) or 1 = active (tentacles extended).

• Cover: 0 = exposed (body fully illuminated, a snail usually on the upper side of a leaf), 1 = partially exposed (body

partially in shade) or 2 = hidden (a snail completely in shade, typically clinging to the underside of a leaf).

Additionally, we recorded

• The distance covered by a snail in the preceding 15 min (to 1 cm).

Analysis

For each variable measured, we summarized all observations of an individual to arrive at a single behavioural score for that individual. We extracted/calculated the maximum and median height above the ground, median illumination, total distance covered and number of observation periods (0-3) during which the movement was recorded, the activity score, ranging from 0 (tentacles hidden at all instances) to 4 (tentacles protruding all the time) and cover score, ranging from 0 (a snail fully exposed at all instances) to 8 (snail always in shade). Next, we calculated the median values of these scores for each group of three simultaneously observed control snails. Finally, we compared these median values with the scores of the concurrently observed infected snails.

To compare the strength of parasite-induced change in particular traits, we used the index of alteration I_a proposed by Cézilly, Favrat & Perrot-Minnot (2013):

$$I_a(d) = [\text{med}(d)_I - \text{med}(d)_u]/\text{IQR}$$

where $\text{med}(d)_I$ denotes the median value of dimension d in infected individuals, $\text{med}(d)_u$ denotes the median value of dimension d in uninfected individuals and IQR is interquartile range of the distribution of d in uninfected individuals, equal to the difference between the upper and lower quartiles ($\text{IQR} = Q_3 - Q_1$).

In agreement with the advice given by Cézilly *et al.* (2013), we used non-parametric tests. All statistical procedures followed the formulae given in the *Electronic Statistics Textbook* (StatSoft, Inc., 2010).

Results

We carried out observations in 25-31 May and 23-26 June 2013, in calm weather (temperatures 12.9-24.4°C). Although the whole study area was densely populated by *Succinea* snails, we found infected snails only in well-lit small glades among trees. We observed 30 infected snails and 90 control ones. They all represented a single species, *Succinea putris*. Similarly, all broodsacs belonged to a single trematode species, *L. paradoxum*.

In consequence of our approach to locate infected snails (with pulsating broodsacs), all the infected snails were active at the onset of observations. In 18 of them, only one pulsating broodsac, in the remaining 12 snails, two pulsating broodsacs, were visible. Most of the infected snails (21/30) remained active throughout the observation period, only one retreated its tentacles after the first record and stayed inactive for the rest of the session. The control snails also remained mostly active; they did not substantially differ from the infected ones in this respect (Fig. 1a, Wilcoxon matched pairs test; $Z = 1.13$, $P < 0.26$).

The snails were recorded 15-125 cm above the ground. The infected ones stayed *c.* 1/3 higher than the control ones, as shown both by distribution of the median heights (Fig. 1b, Wilcoxon matched pairs test; $Z = 3.03$, $P < 0.003$) and of the maximum heights ($Z = 3.03$, $P < 0.003$). Some snails changed their height during the 45 min observation period, approximately equally often up or down (up to 15 cm), there was no difference in this respect between the infected and control ones ($Z = 0.04$, $P \approx 0.96$).

The light intensity values recorded during the observations varied between 80 and 35 500 lx. The infected snails stayed in better lit places than the control ones (Fig. 1c, Wilcoxon matched pairs test; $Z = 3.13$, $P < 0.002$).

The infected snails stayed in more open places than the control ones (Fig. 1d, Wilcoxon matched pairs test, $Z = 2.55$, $P < 0.011$). In fact, 53% (16/30) of the infected versus only 28% (25/90) of the control snails remained fully exposed at all observation instants.

The infected snails were more mobile than the control ones (Fig. 1e, Wilcoxon matched pairs test; $Z = 2.70$, $P < 0.007$), they also moved more persistently ($Z = 3.33$, $P < 0.001$). Only 27% of the infected snails, in contrast to 61% of the control ones, remained stationary during the whole observation period. The snails that actually moved did so in various directions, often drawing convoluted lines, sometimes going back and forth, sometimes making circles.

The effect of infection on alteration of the snail behaviour as expressed by the alteration index $I_a(d)$, widely varied: the activity level was not affected (0); the tendency to stay in the cover (0.7) or in lighter places (0.9) was moderately altered. The strongest affected traits were the height above the ground (1.5) and mobility (3.2). The expression of the particular altered traits was to a large extent independent of one another ($r_s = 0.08$–0.36, $P > 0.05$), only the snails staying higher in the vegetation tended to be in stronger illuminated places ($r_s = 0.56$, $P < 0.002$).

Discussion

Our observations revealed that the snails with pulsating *L. paradoxum* broodsacs behaved differently from their apparently non-infected counterparts. The infected snails differed in all measured traits but the activity level. Moreover, all these changes in the snail behaviour would be beneficial for the parasite. High mobility, combined with dwelling in the well-lit, exposed places, would increase the snails' visibility (detectability) to their potential definite hosts, that is, visually oriented birds. Simultaneously, their habit of staying on the upper surface of leaves, high in the vegetation, would increase their accessibility to the same group of potential hosts. So positioned snails could be approached and attacked from the air, the birds would not have to land and move through dense vegetation to pick the broodsacs. Thus, the observed behavioural changes fulfil the criteria of behavioural manipulation (Poulin, 2010), that is, it seems justifiable to claim that, additionally to its own phenotypic modifications (production of colourful pulsating broodsacs), *L. paradoxum* manipulates also the behaviour of its intermediate *S. putris* host.
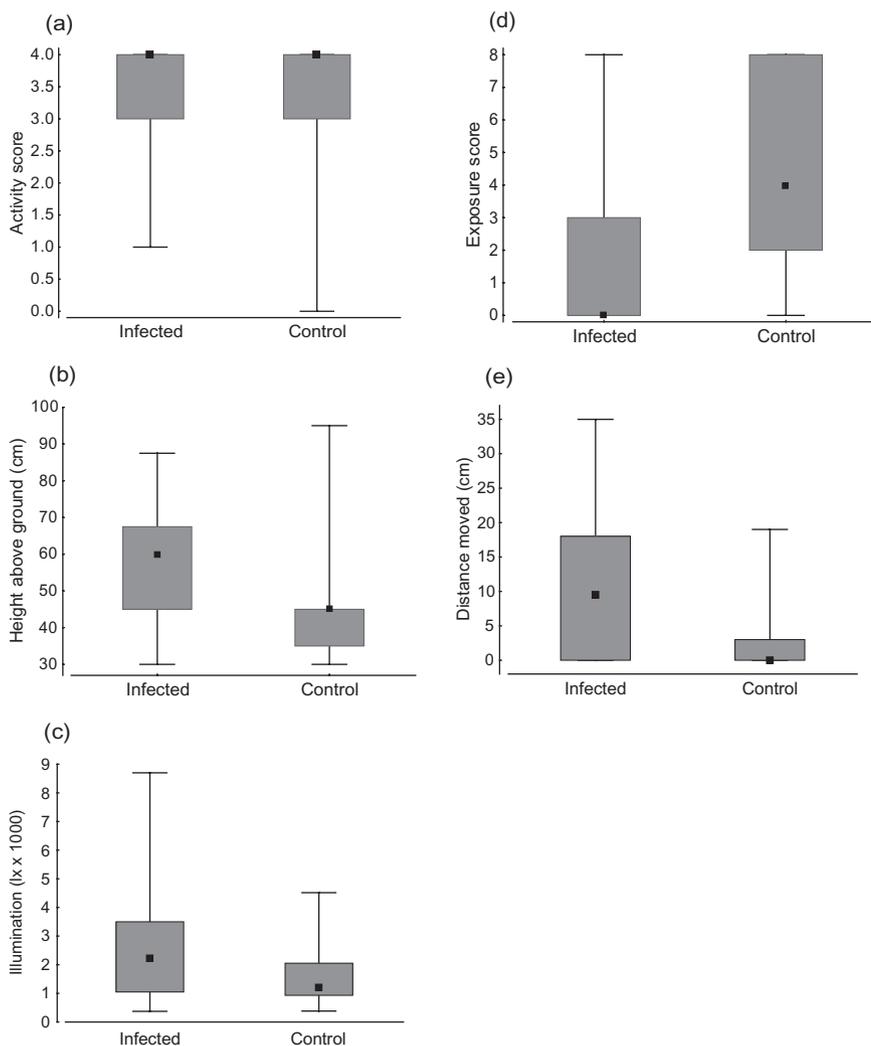
Figure 1 Performance of concurrently observed *Succinea putris* snails containing pulsating *Leucochloridium paradoxum* broodsacs (= infected) and of the apparently non-infected (= control) *S. putris* individuals. Distribution of individual scores of infected snails is compared with the distribution of median scores of three simultaneously observed control snails. Shown are medians (filled squares), 25-75% values (boxes) and ranges (whiskers). See Methods for definitions of variables.

Our findings agree with those of Wesenberg-Lund (1931) on a related species, *L. perturbatum* (also infecting *S. putris*), in that the parasitized snails used to stay in well-illuminated places, on the upper parts of leaves. They sharply differ, though, in respect of the mobility patterns. Wesenberg-Lund (1931) found that 'the parasitized snails are extremely sluggish in all their movements', whereas our data show that the infected snails were much more mobile – actually this was the strongest affected variable of all. We are unable to say whether the divergence between the two studies is due to the real biological interspecific differences or, it rather stems from methodological differences: we observed the snails for short periods, while Wesenberg-Lund generalizations are based on long-term research. The answer has probably to wait until new data, gathered by comparable methods, become available.

It is difficult to compare the *Succinea–Leucochloridium* association with other snail–trematode ones, as – despite enormous economical and health importance of at least some of them – a possibility of manipulation of snail behaviour by these parasites has been relatively rarely studied. Moreover, all studies have been carried out exclusively on freshwater or marine species (reviews in Sorensen & Minchella, 2001; Moore, 2002; followed by Bernot, 2003 and Miura *et al.*, 2006), no studies on land snails seem to exist. Nevertheless, similarly as in our study, changes in behaviour of infected snails, making them more accessible to potential definite hosts, were frequently observed (review in Moore, 2002; Bernot, 2003; Miura *et al.*, 2006). These inferences, though, still require confirmation in the field. Like in numerous other trematodes (review in Sorensen & Minchella, 2001), the *Leucochloridium* sporocysts probably induce castration (but possibly partial and reversible, Wesenberg-Lund, 1931) of their hosts, but in contrast to them (review in Sorensen & Minchella, 2001; Miura *et al.*, 2006), they do not seem to cause their hosts to stunt or grow unusually large (Wesenberg-Lund, 1931).

It seems that the *S. putris–L. paradoxum* association is a unique one. To facilitate transmission, the internal parasites modify, as a rule, the appearance and behaviour of their intermediate hosts (review in Moore, 2002). In this case, both participants 'contribute', the appearance and behaviour of the

parasite and its host are changed. How these multidimensional (Thomas, Poulin & Brodeur, 2010; Cézilly *et al.*, 2013) modifications, encompassing two organisms, combine to facilitate the parasite detection and consumption remains to be discovered. We can only speculate, as it appears that – despite strong prevailing opinions and numerous popular accounts – there is not a single study documenting attacks of definite passerine hosts on snails with broodsacs (Moore, 2002; J. Moore, pers. comm.). Moore (2012) summarizes the current state of knowledge as follows '. . . in the almost 200 years since its description by C. G. Carus in 1835 . . . , both the ecological influence of the parasite and the mechanism by which it accomplishes its visibility have remained more of a puzzle than one might expect . . .'. We think that such a situation is quite embarrassing, and thus, we would like to encourage the readers to undertake studies of this host–parasite association at both the proximate and ultimate levels.

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