The consequences of parasitic infections for host behavioural correlations and repeatability

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Summary

The lifecycles of parasites are often complex, with multiple successive hosts. Many parasites have evolved the ability to modify the behaviour of their present host to increase the chance of transmission to the next host. Few studies to date have considered how a suite of related behavioural traits in the host can be affected by parasitic manipulations. The present study investigated the effect of two trematode parasites, *Coitocaecum parvum* and *Microphallus* sp., on the behaviour of their amphipod host (*Paracalliope fluviatilis*) in a behavioural syndrome framework. This framework focused on the correlations between, and repeatabilities within suites of behavioural traits. Three activity-related behavioural tests were conducted: phototaxis (response to light), horizontal activity, and vertical distribution. Infection by *C. parvum* did not have any significant effect on any of the behavioural traits investigated. However, amphipods infected by *Microphallus* sp. spent less time in the light. Surprisingly, amphipods harbouring *Microphallus* sp. also showed stronger behavioural correlations in general, compared to those of uninfected hosts. Furthermore, the repeatability of the phototaxis test for infected amphipods was lower than that of uninfected amphipods. Given these results, we encourage future studies to use this behavioural syndrome framework.

Keywords: host manipulation, behavioural syndrome, repeatability, animal personality, host-parasite interaction.

Introduction

Parasites often pass through a sequence of two or more hosts, developing as juveniles in intermediate hosts until they reach a definitive host in which they mature as sexually reproductive adults (Kearn, 1998). Transmission of parasites from intermediate to definitive hosts is usually mediated by the lat-

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ter hosts preying on the former hosts (Lafferty, 1999). Many parasites can alter the phenotypes of their intermediate hosts in order to increase their likelihood of predation by and, thus, transmission to, the definitive hosts (Lafferty, 1999; Moore, 2002). There is much empirical evidence for this type of manipulation of intermediate host behaviour, although there are cases where what appears to be manipulation of host behaviour may in fact be by-products of parasitic infections (Poulin, 2000). However, what is common across most studies on host manipulation is a singular focus on the parasitic manipulation of particular behavioural traits rather than pleiotropic consequences of parasitic manipulation on multiple behaviours. In other words, little attention has been paid to how a suite of related behavioural traits in the host (e.g., correlations among behaviours of the hosts) can be affected by parasitic manipulation (but see Benesh et al., 2008). This simplistic approach is a problem not only in studies of the manipulation of host behaviour but also in behavioural studies in general.

However, in the field of behavioural ecology, there has been a recent paradigm shift from looking at behavioural traits separately to looking at suites of related behavioural traits simultaneously (and at how they correlate to each other). This paradigm shift comes from the realization that many behavioural traits of animals (e.g., aggressiveness and boldness) are more repeatable than previously thought and that these traits can be tightly correlated (Sih et al., 2004). Such between-individual consistency of behaviour across different situations and correlations among a suite of behavioural traits are referred to as animal personality or behavioural syndromes (for extended definitions of animal personality see Réale et al., 2007). Five personality traits have been well recognised in the animal personality literature: shyness-boldness (reaction to any risky situation), exploration-avoidance (reaction to a novel situation), activity (general level of activity), aggressiveness (agonistic reaction to conspecifics) and sociability (reaction to the presence or absence of conspecifics, excluding aggression; Réale et al., 2007). One of these traits, the shyness-boldness axis, has been studied in the context of parasitic transmission. Wilson et al. (1993), as a part of their larger study, investigated how bold and shy sunfish might differ in the types of parasites they carried. They observed that sunfish with different degrees of boldness (or shyness) used different habitats, consequently exposing them to different parasite species. In their study, parasite infections were probably a consequence, and not a cause, of particular host behaviours. Furthermore, several other studies have looked at the interaction between parasitism and other behavioural traits such as sociability (Poulin & FitzGerald, 1989; Krause et al., 1999) and aggressiveness (Hamilton & Poulin, 1995). However, few studies have considered how parasite manipulations could change host personality or a suite of behaviours and their correlations.

The present study examines parasite manipulation of hosts in a behavioural syndrome framework. Here, we investigated consistency of and correlations among multiple activity-related host traits, in a system where parasites are transmitted by predation. The study system consists of two species of trematode parasites, *Coitocaecum parvum* (family Opecoelidae) and an undescribed species of the genus *Microphallus* (family Microphallidae) referred to as *Microphallus* sp., and their intermediate host, the freshwater amphipod *Paracalliope fluviatilis*. One of these parasites, *Microphallus* sp., is known to alter the behaviour of its crustacean host in ways that make it more susceptible to predation (Hansen & Poulin, 2005), whereas the other parasite, *C. parvum*, appears incapable of host manipulation (Poulin, 2001). The aim of our work was to examine how parasite infection changes the correlation between three host behavioural traits (phototaxis, horizontal movement and vertical distribution) and the repeatability of these traits. We are well aware of the limitations of our findings, which are observational rather than experimental. Therefore, even when we observe that infected hosts show different patterns of correlation and repeatability from uninfected hosts, we cannot make a causal link between infection and behavioural changes. It is quite possible that innate behavioural differences of hosts can make particular hosts more susceptible to parasite infections as shown in the aforementioned study (Wilson et al., 1993).

Materials and methods

Study species

The host species, a freshwater amphipod (*P. fluviatilis*) is endemic to New Zealand (Sutherland et al., 2007). *Paracalliope fluviatilis* is benthic, and often found grazing on epiphytic algae and diatoms among aquatic plants in both lotic (fast-flowing) and lentic (slow-flowing) systems (Sutherland et al., 2007). Behaviours such as negative phototropism and positive thigmotaxis have been shown in *P. fluviatilis* (Poulin, 2001). *Paracalliope fluviatilis* is used as an intermediate host by at least three different species of parasites, all of which rely primarily on trophic transmission to reach their definitive host

(Lagrue & Poulin, 2008a). Only two of these species (trematodes: *C. parvum* and *Microphallus* sp.) were studied because the third species (the acanthocephalan *Acanthocephalus galaxii*) is very rare (note that none of the amphipods in our study were infected by this species; Lagrue & Poulin, 2008b).

The trematode *C. parvum* goes through a typical 3-host lifecycle (Macfarlane, 1939; Holton, 1984) and uses the common freshwater snail *Potamopyrgus antipodarum* as its first intermediate host. Free-living larvae are produced asexually in snails before emerging from them. Then, the larvae penetrate the second intermediate host, the amphipod *P. fluviatilis*, in which they become enclosed in cysts in the host's body cavity and are referred to as metacercariae. The life cycle is completed when infected amphipods are eaten by their definitive fish hosts (Macfarlane, 1939; Holton, 1984). Some metacercariae are able to develop into adults within the cysts, via progenesis (Macfarlane, 1939; Holton, 1984). These progenetic metacercariae produce viable eggs that are released into the external environment when the amphipod dies (Poulin, 2001; Lefebvre et al., 2005). Progenetic metacercariae, therefore, do not require transmission to a fish, as they attain sexual maturity within the amphipod.

The trematode *Microphallus* sp. is an undescribed New Zealand microphallid species. This parasite uses birds as definitive hosts, aquatic snails as first intermediate hosts, and crustaceans as second intermediate hosts (mostly the isopod *Austridotea annectens*; Hansen & Poulin, 2005). Metacercariae in crustaceans would benefit by altering the behaviour of their host in ways that increase its susceptibility to predation by avian definitive hosts. In another trematode-amphipod system involving related species, a *Microphallus* species induce profound behavioural changes in their amphipod intermediate hosts, causing them to swim at the surface of the water instead of at the bottom of the water column like uninfected amphipods (Helluy, 1984).

Collection of study organisms

Amphipods (*P. fluviatilis*) were collected in January and February 2009 (the middle of the austral summer) from Lake Waihola (40 km south-west of Dunedin, South Island, New Zealand (46°01′S, 170°05′E). They were captured by dragging a dipnet through beds of submerged macrophytes (*Myriophyllum triphyllum*) 2–10 m from the shore (mean depth approx. 0.2 m). Amphipods were returned to the laboratory, where they were stored in a 20-l container of aerated lake water with shoots of *M. triphyllum*, and maintained at room temperature under a natural photo-period.

Experimental procedures

Individual amphipods were used in three successive, distinct behavioural tests, with the order of the tests being randomised. These three tests measured: phototaxis, horizontal movement and vertical distribution (detailed methods below). One observation session involved observing five amphipods for one behavioural trait, then observing another five amphipods for another behavioural trait, then observing yet another five amphipods for the final behavioural trait (therefore, one session involved observing 15 amphipods). Within one session, three observations of the same behavioural traits were undertaken, with approx. 45 min between observations. Each amphipod was used in three sessions, but used in a different test in each session. These observations were performed blind to infection status of individual amphipods, which was subsequently determined following dissection. We carried out 14 sessions in total involving 210 individuals although three died during the tests; thus, we used a total of 207 amphipods. All observation sessions were carried out at room temperature (approx. 20°C) using aerated lake water.

Test for phototaxis

This test measured the response of amphipods to light. A protocol similar to that used in previous studies to observe the effects of parasites on amphipod behaviour was employed (Cézilly et al., 2000; Poulin, 2001). An individual amphipod was placed in each of 5 glass tubes (7.5 cm long, 2.5 cm diameter) filled with water, then sealed with a plastic stopper and placed horizontally. Half of each tube was covered with a dark, opaque material to provide the amphipod with a choice between a light zone and a dark zone of identical volumes. A 60-W lamp, 0.5 m above the experimental glass tubes, provided illumination. After an acclimation period of at least 5 min, the position of each amphipod was recorded at 30-s intervals for 5 min. At each of the 11 recording times, each individual was scored as either 1 (present on the light side) or 0 (not present on light side).

Test for horizontal movement

The level of horizontal activity of each amphipod was measured in this test. An individual amphipod was placed in each of 5 topless shallow plastic containers (25 × 25 × 12 cm) each filled with 200 ml water (9 mm depth), all illuminated by a 60-W lamp set 0.5 m above. Following at least a 5-min acclimation period, at 10-s intervals for 5 min an observation was made of whether each amphipod was actively swimming or not. At each of the 31 recording times, scores for each individual were recorded as either 0 (making no swimming movement) or 1 (actively swimming).

Test for vertical distribution

This test quantified the vertical distribution of the amphipod in a water column. Individual amphipods were placed in clear glass cylinders (20 cm height, 3 cm diameter) filled with 50 ml lake water. To eliminate any phototropism, an opaque box was placed around the cylinders, so that uniform lighting only came from one side of the box that had been removed. A lamp at the same height and 0.5 m from the cylinders provided illumination. At 30-s intervals for 5 min, the height of the amphipod in the cylinder was recorded on a scale of 0 (on the bottom of the cylinder) to 50 (at the surface of the water), for a total of 11 readings. This experiment could also be considered a measurement of geotaxis in each individual.

Parasite identification

At the conclusion of these three behavioural tests, each amphipod was killed in 70% ethanol, rinsed with distilled water, and measured to the nearest 0.02 mm under a dissecting microscope; total body length was taken from the anterior tip of the head (cephalon) to the posterior tip of the tail (telson). Each amphipod was then sexed (males have specialised grasping limbs) and dissected, and the occurrence of the two species of parasites in its body cavity (haemocoel) was recorded along with the number of offspring in the marsupium (brood pouch) of brooding females. The trematode *C. parvum* is characterised by relatively large, oval metacercariae that are enclosed in a delicate and transparent cyst. The presence of the trematode *Microphallus* sp. in the host body cavity was also recorded. It is characterised by a spherical metacercaria surrounded by a thick double-layered cyst (Lefebvre et al., 2005), which was very distinct from the metacercariae of *C. parvum*.

Statistical procedures

All statistical analyses were conducted in the R environment (version 2.9.0; R Development Core Team, 2009). Generalised linear models (GLMs) were used to test for effects of parasite infection on the three behaviours measured in the host, while controlling for the effects of host sex and body size. The response variables for our three statistical models were the measures of phototaxis, horizontal movement and vertical distribution. Each amphipod experienced three repetitions of the same behavioural tests. For the GLM analyses, we pooled these three sessions for each individual by adding their values, which resulted in 207 data points (the same total number of *P. fluviatilis*; the use of the pooled data avoided the problem of pseudo-replication; see Hurlbert, 1984). Data for the light and horizontal activity can be considered as either count or proportion data. We analyzed these data as counts, although analyses using the data as proportion yielded identical results (results not shown). Therefore, for phototaxis and horizontal activity, a log link function was used in conjunction with the quasi-Poisson error structure in the GLMs (Crawley, 2007). On the other hand, the vertical distribution data were log-transformed using ($Y + 0.5$) to approach normality (Yamamura, 1999) and the Gaussian (normal) error was used in the GLM. The predictors of these three GLMs are: infection by *C. parvum*, infection by *Microphallus* sp. (infection was coded as 1 and non-infection as 0), sex of amphipod, and length of amphipod, as well as their second order interactions. Notably, our exploratory analyses showed that none of the second order interactions were statistically significant in any models, so that only the results for the four main effects will be presented.

Pair-wise correlations among the three behaviours ($N = 207$ each) were also calculated using Spearman's rank correlation. The difference between correlation values was assessed with the method using the Fisher R-to-Z transformation (McGraw & Wong, 1996); we also used the same method to assess the difference between repeatability values. Furthermore, repeatability estimates of these three behaviours were calculated using the original data (not the pooled data described above), which included three repetitions for each individual ($N = 621$). We used a linear mixed-effect model (LMM) approach with restricted maximum likelihood (REML, nlme package in R; Pinheiro & Bates, 2000) to estimate repeatability values, following Nakagawa et al. (2007). Statistical significance and 95% confidence intervals were also calculated for repeatability, following the methods described in McGraw & Wong (1996). Note that we provide our raw data as Supplemental Material.

Results

Overall, 62 (30.0%) of amphipods were infected by *C. parvum* and 69 (33.3%) were infected by *Microphallus* sp. out of the 207 amphipods dissected, with the vast majority of infected amphipods harbouring a single metacercaria. Nineteen (9.2%) were co-infected with both trematode species. Amphipod length ranged from 1.01 to 2.65 mm, with a mean of 1.69 mm. Of the 207, 117 (56.5%) were female, while 90 (43.5%) were male.

All the predictors in the three GLM models were statistically non-significant ($p > 0.093$) apart from one factor in the model for the phototaxis behavioural test (Table 1). In this model, *Microphallus* sp. significantly reduced the amount of time spent by amphipods in the light ($p = 0.004$; Figure 1).

There were small but significant positive correlations between phototaxis and horizontal activity, and between phototaxis and vertical distribu-

Table 1. Results from the GLMs concerning host behavioural traits in relation to the two types of parasite infection (*C. parvum* and *Microphallus* sp.), controlling for the effects of sex and body size.

Response	Factor	Estimate (link or transformed scale)	SE	*t* (df = 202)	*p*
Phototaxis	Intercept	1.971	0.686	2.874	0.005
	C. parvum	0.189	0.201	0.938	0.349
	***Microphallus* sp.**	**−0.706**	**0.243**	**−2.906**	**0.004**
	Sex (males)	0.048	0.283	0.171	0.864
	Body size	−0.103	0.458	−0.224	0.823
Horizontal activity	Intercept	3.003	0.430	6.985	<0.001
	C. parvum	−0.109	0.134	−0.810	0.419
	Microphallus sp.	0.111	0.127	0.878	0.381
	Sex (males)	0.128	0.176	0.730	0.466
	Body size	−0.001	0.284	−0.003	0.998
Vertical distribution	Intercept	4.544	1.247	3.645	<0.001
	C. parvum	0.637	0.377	1.690	0.093
	Microphallus sp.	−0.537	0.371	−1.450	0.149
	Sex (males)	0.284	0.511	0.557	0.578
	Body size	−0.962	0.826	−1.165	0.245

The sex effect here is the male effect, the female effect is included in the intercept and the significant main effect is in boldface.

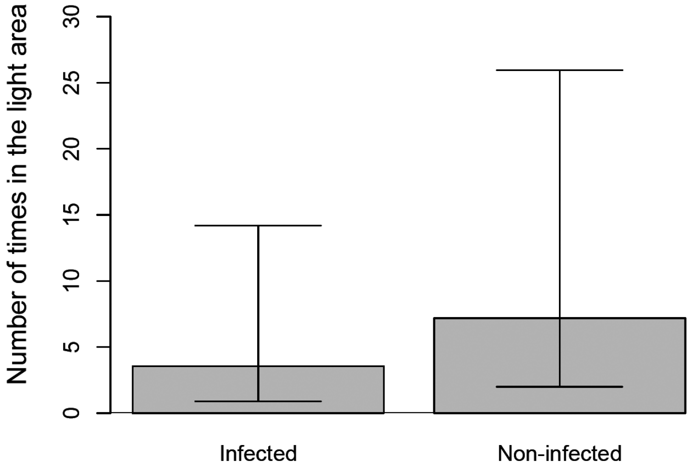

Figure 1. The number of times the amphipods were found in the light area when they were infected by *Microphallus* sp. ($N = 69$) and not infected by *Microphallus* sp. ($N = 138$) along with the 95% confidence intervals (out of a total number of 33 recordings for each amphipod); note the original confidence intervals were calculated in the log-link scale and converted to the count scale.

tion ($r = 0.17$ and 0.15, respectively, Table 2). Note that Cohen's effect size benchmarks for correlation coefficient are $r = 0.1, 0.3$ and 0.5 being considered as 'small', 'medium' and 'large', respectively. The correlation between horizontal activity and vertical distribution was weak and non-significant. Because of the significant effect of *Microphallus* sp. on the amount of time the amphipods spent in the light, the data were split to calculate separate correlations for individuals infected and non-infected by *Microphallus* sp. When the amphipods were infected by *Microphallus* sp., host behavioural correlations between phototaxis and horizontal activity and between phototaxis and vertical distribution were medium and significant (the correlation between horizontal activity and vertical distribution was still non-significant). On the other hand, when the amphipods were not infected by *Microphallus* sp., the corresponding correlations were both small and non-significant. Notably, none of the differences among corresponding pair-wise correlations were significant ($p > 0.12$).

Repeatabilities of all the behavioural traits were very high ($R > 0.52$) and statistically significant ($p < 0.001$; calculated from the data for all amphipods; $N = 207$, Figure 2). We also calculated repeatability estimates for both *Microphallus*-infected and uninfected individuals because of the

Table 2. Spearman rank correlations, r_s (p values) in the upper diagonals and their 95% confidence intervals in the lower diagonals among the three host behavioural tests for the total dataset, and separately for individuals infected and not infected by the trematode *Microphallus* sp.

	Total ($N = 207$)			Infected ($N = 69$)			Non-infected ($N = 138$)		
	Phototaxis	Horizontal	Vertical	Phototaxis	Horizontal	Vertical	Phototaxis	Horizontal	Vertical
Phototaxis	—	**0.17 (0.02)**	**0.15 (0.03)**	—	**0.27 (0.03)**	**0.29 (0.02)**	—	0.12 (0.15)	0.07 (0.43)
Horizontal	**0.03–0.30**	—	0.10 (0.17)	**0.03–0.47**	—	0.14 (0.24)	−0.04–0.28	—	0.09 (0.30)
Vertical	**0.01–0.28**	−0.04–0.23	—	**0.06–0.49**	−0.10–0.37	—	−0.10–0.23	−0.08–0.25	—

The statistically significant results are in boldface.

Table 3. Estimates of between- and within-individual variance from linear mixed-effect models for the three host behavioural tests for the total dataset, and separately for individuals infected and not infected by the trematode *Microphallus* sp.

	Total ($N = 207$)			Infected ($N = 69$)			Non-infected ($N = 138$)		
	Phototaxis	Horizontal	Vertical	Phototaxis	Horizontal	Vertical	Phototaxis	Horizontal	Vertical
Between-individual variance	0.781	1.752	2.868	0.322	1.919	2.341	0.970	1.676	3.046
Within-individual variance	0.413	0.786	2.623	0.451	0.718	2.972	0.394	0.821	2.449

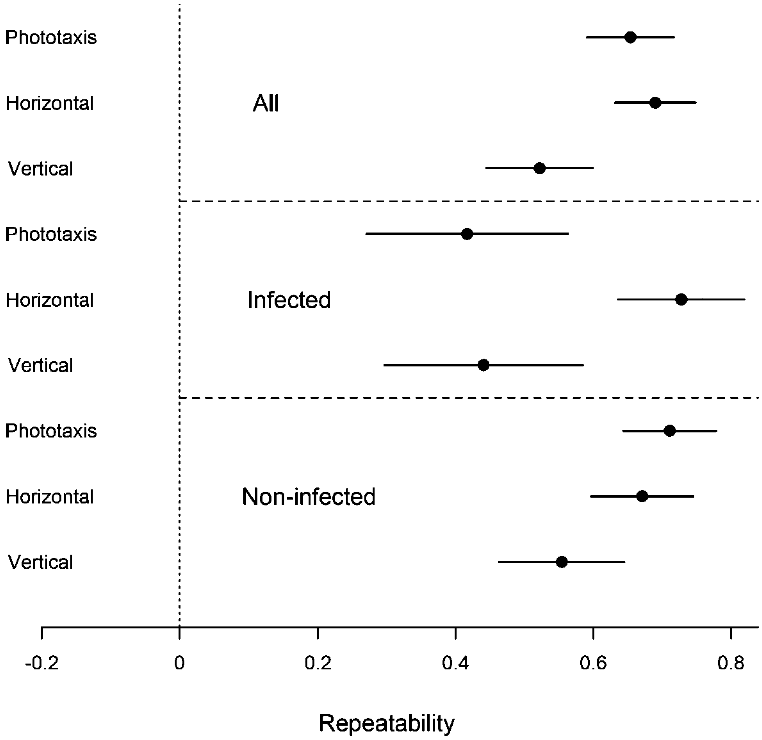

Figure 2. Repeatability estimates and their 95% confidence intervals for the three behavioural tests (phototaxis, horizontal activity and vertical distribution) in the three datasets: All (all amphipods included; $N = 207$ with 3 repeats each), Infected (the amphipods infected by *Microphallus* sp.; $N = 69$) and Non-infected (the amphipods not infected by *Microphallus* sp.; $N = 138$); all repeatability values are significantly larger than zero ($p < 0.001$).

significant effect of that parasite on phototaxis. The repeatability value for phototaxis was significantly smaller when amphipods were infected by *Microphallus* sp. than when not infected ($Z = -5.42$, $p < 0.001$). On the other hand, the repeatability estimates for horizontal activity and vertical distribution were not significantly different when comparing behavioural tests between infected amphipods and non-infected ones ($Z = 1.85$, $p = 0.065$, $Z = -1.74$, $p = 0.008$, respectively). This significant decline in the repeatability value for phototaxis in the infected hosts was driven by a decrease in the between-individual variance rather than an increase in the within-individual variance (Table 3).

Discussion

Does parasitic infection affect host behaviour?

The results showed that *C. parvum* did not have any significant effect on any of the behavioural traits investigated (phototaxis, horizontal activity, vertical distribution). These results partially parallel the results of Poulin (2001), who found no significant differences in his tests for photophilia and for activity between infected and non-infected amphipods. As mentioned in the Methods section, *C. parvum* are able to grow and reproduce inside the amphipod, rather than requiring their final host (fish) to complete their life-cycle (Poulin, 2001; Lefebvre et al., 2005). This strategy, known as progenesis, is a more radical way to counteract the low odds of trophic transmission than host manipulation. Poulin (2001) explains that the presence of at least one other predatory fish species unsuitable as a definitive host in this system may reduce the efficiency of host manipulation by *C. parvum*, as host manipulation may increase predation of the amphipod by an unsuitable host, and decrease the survival of the parasite. Poulin (1994) and Brown (1999) argue that many parasites with complex life cycles such as *C. parvum* are not manipulators because their alternative methods of transmission and/or reproduction reduces the relative pay-offs of host manipulation. Amphipods infected by *Microphallus* sp. spent significantly less time in the light during the phototaxis test than uninfected ones. However, we expected an increase in *positive* phototaxis and high scores in vertical distribution for *Microphallus*-infected amphipods to make them more vulnerable to bird predators. In fact, another *Microphallus* species (*Microphallus papillorobustus*) modifies the behaviour of its intermediate host amphipod (*Gammarus insensibilis*) by employing positive phototaxis, negative geotaxis and an aberrant evasive behaviour (Helluy, 1984). Infected individuals are twice as likely to be preyed on by birds (their final host) than non-infected ones (Helluy & Thomas, 2003). Perhaps the site of encystment provides an explanation for the different manipulative ability of *M. papillorobustus* and of our study species. *Microphallus papillorobustus* encysts in the nervous system of amphipods and is, therefore, well-placed to modify the behaviour of the host by producing or regulating neurotransmitters such as serotonin, dopamine, and octopamine (Øverli et al., 2001). In contrast, the *Microphallus* species we studied encysts in the body cavity of its host. Our study species can nonetheless alter the behaviour of its other intermediate host, the isopod *Austridotea annectens*, by increasing its activity level and reducing its evasive responses (Hansen & Poulin, 2005). It is possible that the parasite is better able to manipulate one of its intermediate hosts than the other, perhaps because the isopod was historically the ancestral host. In any event, further work is required to investigate whether or not the negative phototaxis observed here in infected amphipods could increase the transmission of *Microphallus* sp.

Behavioural correlations, repeatability and parasitic infection

Without considering the effects of *Microphallus* sp. infection, only weak pair-wise correlations were found among the three behavioural traits ($0.10 \leqslant r \leqslant 0.17$). Furthermore, once we remove the effect of *Microphallus* sp. infection (i.e., just looking at the uninfected individuals), these correlations became even weaker and non-significant ($0.07 \leqslant r \leqslant 0.09$). These results indicate that these three activity-related traits were, somewhat surprisingly, independent of each other, at least in 'normal' and healthy (uninfected) amphipods. Notably, amphipods infected by *Microphallus* sp. displayed stronger behavioural correlations in general ($0.14 \leqslant r \leqslant 0.29$). It has been shown that parasitic infections can change the mean value of behavioural traits as well as their variance (reviewed in Poulin & Thomas, 1999). Therefore, it may not be surprising that parasitic infection can induce or strengthen correlations among behavioural traits. Analogous results have been found in behavioural syndrome studies, which are useful to put our results into perspective. Dingemanse et al. (2007) found that predation pressure predicted whether there were correlations between boldness and aggressiveness in natural populations of sticklebacks ($-0.10 \leqslant r \leqslant 0.44$; the results indicated that predation induced behavioural correlations). Furthermore, Bell & Sih (2007) experimentally demonstrated that predation by trout could generate a correlation between boldness and aggressiveness ($r = 0.46$) in a population of sticklebacks where that correlation was originally weak ($r = 0.14$; see also Bell & Stamps, 2004, where changes in the correlation between boldness and aggressiveness were monitored over developmental stages). These two studies suggested that stress incurred from predation pressure could be a cause of the observed behavioural correlation. Therefore, similar stress responses in the infected hosts can explain our finding of the increased behavioural correlations among the activity-related traits. Although it is difficult to envisage a physiological or neurobiological mechanism for the increased correlations found in our study, such parasite mediated behavioural correlations will be an interesting avenue of future research.

Repeatability of each behavioural trait was high ($0.52 \leqslant R \leqslant 0.69$) before considering the *Microphallus* sp. infection (Figure 2). The parasitic infection had a significant effect on repeatability of phototaxis (whose mean values were significantly altered by *Microphallus* sp. infection; Figures 1 and 2). This reduction in the repeatability estimates of phototaxis is due to a reduction in the variability of the response to light between the infected individuals. Such reduction in the variance may be expected if *Microphallus* sp. indeed manipulates the phototaxis behaviour of the amphipods, by homogenising the amphipods' response to light. Interestingly, a meta-analysis of 114 studies containing 759 estimates of repeatability for a wide range of behavioural traits found the meta-analytic mean repeatability to be 0.37 (Bell et al., 2009). Thus, although amphipods infected by *Microphallus* sp. may have reduced repeatability values for phototaxis and vertical distribution ($R = 0.42$ and 0.44, respectively), these values are still higher than the average repeatabilities for a range of behavioural traits. As far as we know, it is the first demonstration that parasitic infection can change repeatability values.

Conclusion

We investigated the effect of two trematode parasites, *C. parvum* and *Microphallus* sp., on the behaviour of their second intermediate amphipod host (*Paracalliope fluviatilis*) in a behavioural syndrome framework. Three activity-related behavioural tests were conducted: phototaxis (response to light), horizontal activity, and vertical distribution. It should be noted that this study did not investigate behavioural syndromes as such, but examined parasite host manipulation in a behavioural syndrome framework. This framework focuses on the correlations between, and repeatabilities within suites of behavioural traits (Sih et al., 2004). Although the experimental infection of laboratory-bred amphipods would ultimately be needed, valuable insights into how parasites affect the correlations and repeatabilities of behavioural characters of their hosts were obtained. We encourage future studies on parasitic manipulations of hosts to employ a behavioural syndrome framework to achieve greater insight into this phenomenon.

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