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Transmission of parasitic mites (*Riccardoella oudemansi*) between limacid slug hosts: the role of parasite and host behaviour

Mark J. Roper[1] · Ruth E. Arnold[1] · Kieran E. Storer[1] · Jonathan P. Green[1]

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Abstract

Transmission between hosts is crucial to the growth, development and reproduction of many parasites. As a consequence, parasites are under selection to maximise transmission success and exhibit many behavioural and morphological adaptations that allow detection of, and movement between, hosts. However, transmission success is not determined by parasites alone, but is also shaped by host behaviours. Often, host behaviours function to minimise the risk of exposure to parasites; in some cases, however, host behaviours may be manipulated by parasites to increase transmission success. In this study, we investigated transmission of the parasitic mite *Riccardoella oudemansi* between slug (*Limacus maculatus*) hosts, considering the role of both host and parasite behaviour in determining transmission success. Host-host transmission occurred when slugs were in physical contact, but mites were also capable of moving across the substrate to locate new hosts, a process facilitated by mucus trails. We found no strong evidence that slugs avoid parasitised conspecifics, or that mites manipulate slug behaviour to increase transmission. Finally, mites showed a preference for the mucus of parasitised slugs, but did not discriminate between mucus from their own host and another parasitised slug. A general preference for mucus from parasitised slugs is likely to be important in encouraging mites to remain in close contact with their host and may also facilitate host-switching and outbreeding. We encourage further study of parasitism by *Riccardoella* in limacid slugs, where cross-species variation in host social behaviour may drive differences in the rate and success of parasite transmission across slug species.

Keywords *Riccardoella* · *Limacus* · Parasite · Mite · Slug · Host

1 Introduction

Transmission success is a fundamental determinant of fitness in parasitic organisms and selection is therefore expected to act strongly on strategies for ensuring efficient transmission between hosts (Galvani 2003; Poulin 2007). Such strategies may include host-seeking behaviours by parasites (Combes 1991), as well as behavioural changes in the host engineered by the parasite to encourage host-host contact (Moore 2002). For their part, hosts are also often under strong, reciprocal selection to minimise the costs of parasitism, which can often result in the evolution of strategies for detecting and avoiding parasites (Sarabian et al. 2018). Thus, traits expressed by both parasites and hosts can interact to determine the efficacy of parasite transmission and the resulting distribution of parasites within host populations (Galvani 2003; Buck et al. 2018).

Terrestrial pulmonate gastropods are hosts to parasitic mites of the genus *Riccardoella* (Fain 2004). *Riccardoella* mites feed on haemolymph from the host's lung epithelium within the pallial cavity (Baker 1970), but otherwise spend a significant amount of time moving over the surface of the host's body. Of the six *Riccardoella* species currently known to parasitise gastropods (Waki et al. 2021), most research has focused on the interaction between *R. limacum*, a snail parasite, and two helicid snail hosts, *Arianta arbustorum* and *Cornu asperum* (e.g. Graham 1994; Graham et al. 1996; Baur & Baur 2005; Schüpbach & Baur 2008a, 2008b, 2010a, 2010b; Wacker 2008). In these species, mite infestations are associated with reduction in growth and survival (Schüpbach & Baur 2010b; Wacker 2008), as well as a decline in fecundity (Schüpbach & Baur 2008b) and a

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✉ Jonathan P. Green
jonathan.green@biology.ox.ac.uk

[1] Department of Biology, University of Oxford, 11a Mansfield Road, OX1 3SZ Oxford, UK

shift in sex allocation (Haeussler et al. 2014). In addition, behavioural effects of parasitism have also been reported, including a reduction in overall activity (Graham et al. 1996; Schüpbach & Baur 2008b), and feeding activity in particular (Wacker 2008). In addition to snails, a number of slug species are also parasitised by *Riccardoella* mites, but to date this parasitic relationship has received very little attention (but see Akimov & Zabludovska 2009).

Transmission of *Riccardoella* mites between hosts is known to occur when hosts are in physical contact, but can additionally occur when mites leave their host and traverse the substrate to seek a new host (Schüpbach & Baur 2008a, 2010a). In the latter case, it has been suggested that mites may use mucus trails to locate new hosts (Turk and Phillips 1946; Schüpbach & Baur 2008a). Investigating transmission of the mite *R. limacum* between snail (*Arianta arbustorum*) hosts, Schüpbach & Baur (2008a) found that freely-moving mites were more likely to remain on mucus deposited by their host than on a substrate without mucus, but only when the mucus was fresh. This finding may suggest that mites utilise mucus trails to locate new hosts; alternatively, the attraction to fresh mucus from a mite's own host may ensure that they remain in close contact with their host, relocating it rapidly if they become separated. Distinguishing between these possibilities requires testing preferences of mites for mucus from their own host and mucus from a second host. At the same time, it is also unclear whether mites seeking new hosts prefer unparasitised slugs or slugs already infected with mites. A preference by parasites for healthy (i.e. unparasitised) hosts is observed in a range of host-parasite systems and is commonly interpreted as a strategy by which parasites can escape competition with conspecifics for resources (e.g. Grewal et al. 1997; Tamò et al. 2006; Allan et al. 2009). A reduction in feeding success at high densities has been observed in other hematophagous ectoparasites (Hawlena et al. 2007; Jones et al. 2015) and could plausibly occur in *Riccardoella* when colonies are very large, driving searching for new, unparasitised hosts. Conversely, a preference for parasitised hosts may facilitate outbreeding by parasites (see e.g. Gómez-Díaz et al. 2007; van Schaik et al. 2014), or may reflect avoidance of hosts with strong anti-parasite (e.g. immune) defences.

Here, we explore transmission behaviour in the mite *R. oudemansi*, which parasitises a number of terrestrial slug species (Zabludovskaya and Badanin 2010). We studied transmission of mites between green cellar slugs, *Limacus maculatus*; to our knowledge, this is the first detailed examination of parasitism by *Riccardoella* mites in this species. We first confirmed that transmission can occur during physical contact between hosts, as reported for *R. limacum* infecting snails, before then investigating transmission success via soil in the presence and absence of mucus. Finally, we used choice assays to investigate mite responses to determine, firstly, whether mites prefer mucus from parasitised or unparasitised hosts. Having found evidence that mites prefer mucus from parasitised hosts, we then secondly asked whether mites showed a general preference for mucus from parasitised slugs by comparing responses to mucus from the mites' own host to that of a second parasitised slug.

In a second set of experiments, we asked whether mite transmission is influenced by host behaviour. Among terrestrial gastropods, cues in mucus are known to facilitate species and sex recognition (Ng et al. 2013), while in Atlantic salmon (*Salmo salar*) and common periwinkles (*Littorina littorea*) mucus provides cues to parasitism by copepods and trematodes respectively (Erlandsson & Kostylev 1995; Difford et al. 2022). It is thus plausible that mucus of *L. maculatus* could provide information about the infection status of conspecifics. To investigate this possibility, we tested whether slugs avoid substrate and shelters containing mucus from parasitised conspecifics. Since it is known that parasitism of other gastropod species by *Riccardoella* mites is associated with changes in behaviour (Schüpbach & Baur 2008b; Wacker 2008), we compared responses to mucus in both unparasitised and parasitised slugs to determine whether the strength of any response was dependent on an individual's infection status. Among parasitised individuals, a stronger attraction to conspecific mucus, including attraction to mucus of parasitised conspecifics, would be consistent with manipulation of host behaviour by mites to increase successful transmission and outbreeding. Stronger attraction to unparasitised slugs may point to behavioural manipulation to increase transmission to healthy individuals to take advantage of more resources.

2 Materials and methods

2.1 Study population

L. maculatus is a large limacid slug that is widely distributed across Europe and inhabits a variety of urban environments (Kerney 1999). Captive populations were established in May 2017, September 2021 and September 2022 from parasitised and unparasitised adults collected in Oxford and Banbury (Oxfordshire, UK). We assumed that all mites found on slugs were *R. oudemansi*, since this is the only *Riccardoella* species known to parasitise slugs, including *L. maculatus*, in Europe (Zabludovskaya and Badanin 2010; Stojnić et al. 2016). Of the other parasitic *Riccardoella* species, all but one are specialist parasites of snails (Graham et al. 1993; Fain 2004; Waki et al. 2018, 2019; Waki & Shimano 2019), while the other slug specialist, *R. novaezealandidae*, is known only from the South Pacific (Fain and

Barker 2004). Determining an individual's infection status with 100% certainty would have necessitated dissection, which was incompatible with the goals of the study. Instead, we used the screening method of Baur & Baur (2005), inspecting the body surface and pallial cavity at the time of capture and each day for the following three days. Accuracy of this method for determining an individual's infection status has been estimated at 87.5% (Baur and Baur 2005). Slugs designated as "unparasitised" were also checked for mites immediately prior to use in experiments. For the duration of the study, slugs were housed individually in transparent plastic containers (16.8 × 10 × 4 cm) with perforated lids. A soil substrate was provided in each container, which was kept moist. Soil used in housing and in experimental arenas was first microwaved to kill any free-living mites that might have been present. Fresh lettuce and apple was provided *ad libitum* as food and containers were cleaned regularly. Following completion of the experiments, individuals were released. The experiments described below were carried out during daylight hours. Slugs were used repeatedly across experiments, but were only used once within an experiment.

2.2 Mite transmission between hosts

To determine whether transmission of mites is possible during physical contact between slugs, 3 pairs of slugs (1 parasitised and 1 unparasitised) were placed together in a 10 × 10 cm arena and observed for 10 min.

Next, to determine whether transmission of mites can occur in the absence of host-host contact, one parasitised and one unparasitised slug were placed in an arena (16.8 × 10 × 4 cm) with a soil substrate. Two types of trial were run, each with 15 pairs of slugs. In the first, slugs were placed on either side of a central barrier consisting of a single strip of cardboard (0.5 cm width) punctured with numerous holes approximately 1 cm in diameter that were large enough for mites to pass through. This design, which prevented physical contact between slugs but allowed contact between their mucus, aimed to test whether mites were able to move between hosts by walking on mucus. In the second set-up, slugs were placed on either side of a central pair of barriers (as above, cardboard strips of 0.5 cm width with numerous holes) placed 2 cm apart. This design prevented contact between slugs or mucus, requiring mites to traverse bare soil in order to move between hosts. Trials were run for 72 h, after which time unparasitised slugs were checked for the presence of mites using the screening method described above. Transmission was deemed to have occurred if at least one mite was found on the previously unparasitised slug. The proportion of trials in which transmission occurred was compared between the two trial types using a generalized linear model (GLM) with binomial errors with transmission success (yes/no) as the binary response and trial type as the predictor.

Finally, two experiments were performed to explore mite responses to mucus. In both experiments, subjects were mites collected from parasitised slugs in our captive population. In the first experiment, individual mites were presented with the choice of mucus from a parasitised and an unparasitised slug, applied in equal amounts to each half of a 2 × 2 cm arena (microscope slide mounted on card). Across trials, parasitised and unparasitised mucus was distributed evenly between the left and right sides of the arena. At the start of a trial, a mite was transferred to the centre of the arena using a fine paint brush and the amount of time that it spent on each side of the arena was recorded over three minutes. A total of 48 mites were tested, 6 mites each from 8 different parasitised slugs. In half the trials, mites were placed on the arena immediately following application of the mucus (fresh mucus treatment), while in the other half mites were placed on the arena four hours later once the mucus had fully dried (old mucus treatment). For each trial, mucus was collected from a different pair of parasitised and unparasitised slugs. To test whether the infection status of the donor and/or the freshness of the mucus influenced the time that mites spent on mucus, the time that mites spent on the left side of the arena was modelled using a linear mixed-effects model (LMM). Infection status of the donor (parasitised or unparasitised) and freshness of the mucus on the left side of the arena were included as fixed effects, together with the interaction between infection status and freshness. To account for the potential similarity in response from mites sharing the same host, the identity of the slug from which mites were taken was included as a random effect.

In the second experiment, individual mites were presented with the choice of fresh mucus from their own host and a second parasitised slug. Trials were carried out as described above. A total of 46 mites were tested from 14 different parasitised slugs (1–7 mites per slug). Mites from individual slugs were presented with mucus from unique pairs of slugs. To test whether the identity of the parasitised slug influenced the time mites spent on mucus, the time that mites spent on the left side of the arena was modelled using an LMM with donor type (own host or another parasitised slug) as a fixed effect. To account for the potential similarity in response from mites sharing the same host, the identity of the slug from which mites were taken was included as a random effect. Across both experiments, individual mites were only used once.

2.3 Host responses to mucus

To determine whether slugs avoid parasitised conspecifics, responses of slugs to mucus of parasitised and unparasitised conspecifics were examined in two experiments. In the first experiment, responses to mucus on a soil substrate were tested in a 10 × 10 cm arena containing a layer of soil. Prior to the trial, a donor slug was placed in one half of the arena and allowed to move freely for 5 min to deposit mucus. Across trials, equal numbers of donors were placed in the left and right sides of the arena. The donor slug was then removed and a second slug (the subject) was then immediately placed at the centre of the arena and allowed to move freely around the entire arena for 10 min. To encourage movement by both slugs, the arena was shaded. The infection status of the donor and subject were varied in a full factorial design (both unparasitised, both parasitised, donor unparasitised and subject parasitised, donor parasitised and subject unparasitised), with each combination repeated 12 times, resulting in a total of 48 trials. A different donor and subject were used in each trial. The number of subjects spending more time on the side of the arena containing mucus was compared with the number spending more time on the side of the arena without mucus using a binomial test. Whether the subject spent more time on the side of the arena containing mucus from the donor (yes/no) was analysed using a GLM with binomial errors with infection status of the donor, infection status of the subject and their interaction as predictors.

In the second experiment, responses to mucus in shelters were tested, again using a 10 × 10 cm arena containing a layer of soil. Two opaque plastic cups (diameter: 4 cm) placed 1.5 cm apart on the soil surface provided shelters, which slugs could access through a small square hole at the base. Immediately prior to a trial, a single donor slug was placed in each cup and allowed to move freely for five minutes to deposit mucus. In each trial, one parasitised and one unparasitised slug were used as donors. A different pair of donor slugs was used in each trial. After five minutes, the donor slugs were removed and the cups placed side-by-side in the arena. A third individual (the subject) was then placed at the centre of the arena, midway between and facing the two cups and allowed to move freely within the arena for 15 min. To encourage subjects to use the shelters, the arena was uniformly illuminated by a 35-watt lamp from a height of 30 cm. Forty-six trials were run; in half, the subject was parasitised, while in the other half an unparasitised subject was used. The number of subjects that first entered the shelter containing mucus deposited by the parasitised donor was compared with the number first entering the shelter containing mucus deposited by the unparasitised donor using a binomial test. Whether a subject subsequently left the first shelter it entered (yes/no) was analysed using a GLM with binomial errors, with infection status of the donor, infection status of the subject and their interaction as predictors.

2.4 Statistical analyses

All analyses were performed in R version 4.0.3 (R Core Team 2020). LMMs were carried out using the *lme* function from the *nlme* package (Pinheiro et al. 2021). For linear models, test statistics (χ^2 for GLMs and log-likelihood [L] ratios for LMMs) and *P* values for each predictor were calculated by comparing the full model to a second model without the predictor, having first removed any nonsignificant interaction terms. All tests were two-tailed and effects were considered to be significant at $P < 0.05$.

3 Results

3.1 Mite transmission between hosts

Direct movement of mites from infected to uninfected individuals during physical contact was observed in all three pairs. When physical contact between hosts was prevented, transmission still occurred, however, and tended to occur more frequently via mucus (9/15 trials) than bare soil (3/15 trials; $\chi^2_1 = 3.45$, $P = 0.06$). When presented with mucus from parasitised and unparasitised slugs, mites showed a preference for mucus from parasitised hosts, but only when this mucus was fresh (host infection status * mucus age: $L_1 = 5.84$, $P = 0.02$, $n = 48$; Fig. 1). Excluding 7 mites that spent the entire time on one side of the arena produced similar results ($L_1 = 4.25$, $P = 0.04$, $n = 41$). While mites showed a preference for mucus from parasitised hosts, they did not discriminate between mucus from their own host and mucus from another parasitised slug ($L_1 = 1.10$, $P = 0.29$, $n = 46$; Fig. 2). Similar results were obtained when excluding 4 mites that showed no searching behaviour ($L_1 = 1.31$, $P = 0.25$, $n = 42$) and when excluding a further 20 mites that spent the entire time on one side of the arena ($L_1 = 0.15$, $P = 0.70$, $n = 22$).

3.2 Host responses to conspecific mucus

Responses of slugs were recorded to conspecific mucus on soil and in shelters. Slugs tended to spend more time on the side of the arena where a donor slug had previously deposited mucus (31/48 trials, $P = 0.06$), but this preference was not influenced by the infection status of the donor ($\chi^2_1 = 0.84$, $P = 0.36$) or the subject ($\chi^2_1 = 0.84$, $P = 0.36$; donor * subject infection status: $\chi^2_1 = 2.17$, $P = 0.14$; Fig. 3A). In the second assay, slugs entered one or more shelters in 44/46 trials. Subjects first entered the shelter previously occupied by a parasitised donor in 19 trials and the shelter occupied by a parasitised donor in 25 trials (43% vs. 57%, $P = 0.45$). In 20 trials (45%), the subject left the first shelter it entered. The

Fig. 1 A: Time spent by mites on mucus from parasitised (P) or unparasitised (UP) slugs when mucus was either fresh or old. Data are model-predicted means ± 95% confidence intervals (CIs). B: Difference in time spent on mucus (parasitised – unparasitised) when mucus was either fresh or old. The grey dashed line indicates a difference of zero. Data are means ± 95% CIs. Points are jittered to facilitate visualisation of the data

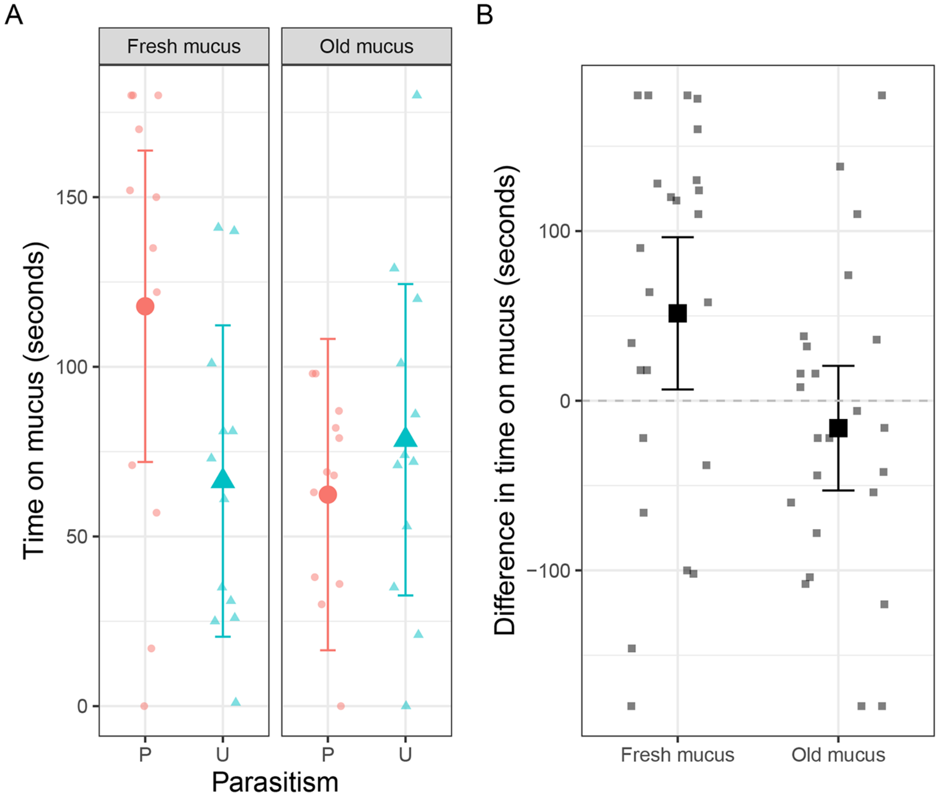
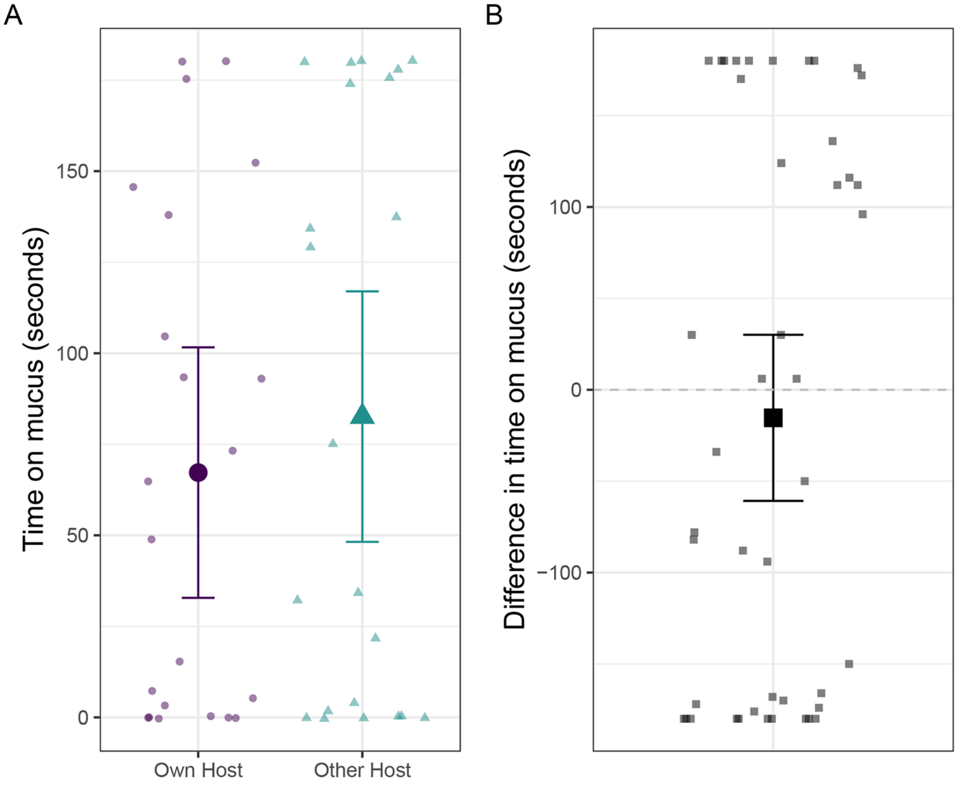


Fig. 2 A: Time spent by mites on mucus from their own host slug and from another parasitised slug. Data are model-predicted means ± 95% CIs. B: Difference in time spent on mucus (own host – other parasitised slug). The grey dashed line indicates a difference of zero. Data are means ± 95% CIs. Points are jittered to facilitate visualisation of the data



infection status of the subject did not influence whether it left the shelter ($\chi^2_1 = 0.07$, $P = 0.78$; subject status * shelter

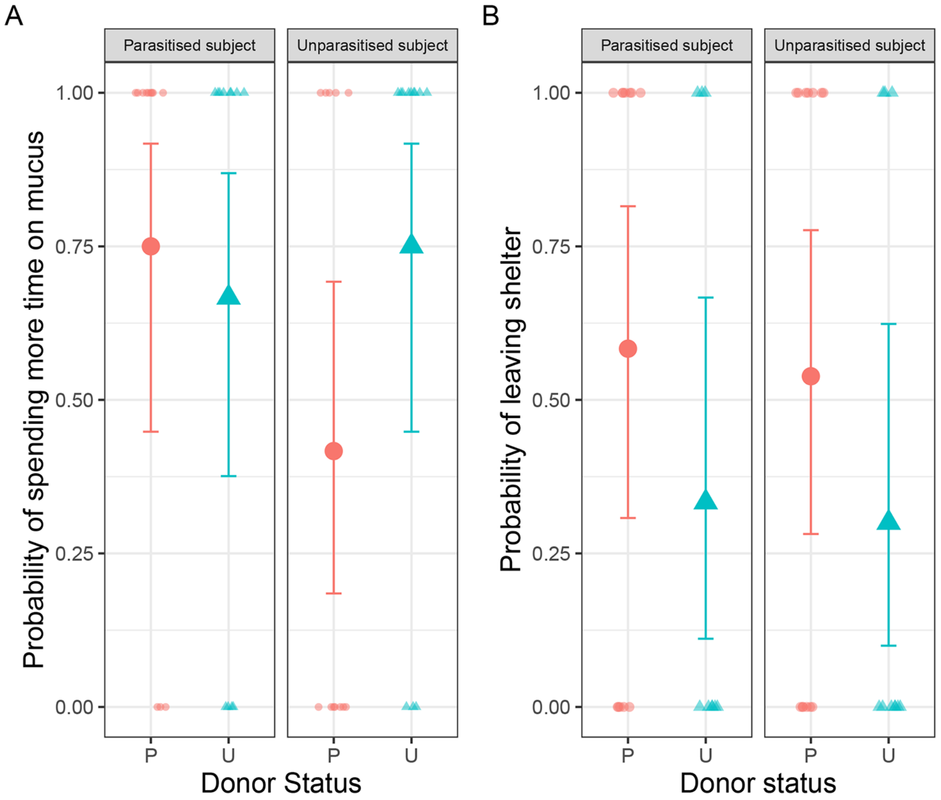


Fig. 3 A: Probability that slugs spend more time on the side of the arena containing mucus trails in relation to their own parasitism status and the parasitism status (P = parasitised, U = unparasitised) of the mucus donor. B: Probability that slugs leave the first shelter they enter in relation to their own parasitism status and the parasitism status (P = parasitised, U = unparasitised) of the mucus donor. Data are model-predicted means ± 95% CIs. Points are jittered to facilitate visualisation of the data

status: $\chi^2_1 = 0.00$, $P = 0.98$; Fig. 3B). Slugs tended to leave the shelter more often if it had been occupied by a parasitised versus unparasitised donor (14/25 trials [56%] vs. 6/19 trials [32%]), but this difference was not significant ($\chi^2_1 = 2.64$, $P = 0.10$; Fig. 3B).

4 Discussion

Although *Riccardoella* mites are common parasites of numerous terrestrial gastropod species, including both slugs and snails, detailed analysis of this host-parasite relationship has so far been restricted to two snail species, *A. arbustorum* and *Cornu asperum* parasitised by *R. limacum*. Consequently, much remains to be learned about the interaction between gastropods and *Riccardoella* mites, including the impact of this symbiosis on the life history, behaviour, and ecology of each party. Here, we investigate parasitism of a slug host by *Riccardoella* mites, focusing on routes of transmission and the impacts of parasite and host behaviour on transmission success.

As in snails, transmission of mites in *L. maculatus* occurs readily when hosts are in physical contact. Prolonged physical contact occurs during copulation in *Limacus* species (Karlin and Bacon 1961), suggesting that mites are likely to be transmitted sexually, as has previously been hypothesised (Turk and Phillips 1946). In the case of *L. maculatus*, social transmission likely also occurs during day-time rest, when individuals huddle together in close contact (Cook 1981b). If parasitism is costly to host slugs, selection should favour strategies that limit contact, either sexual or social, with infected individuals. In snails, parasitism by *Riccardoella* mites is associated with a reduction in growth, reproductive output, and survival (Graham 1994; Graham et al. 1996; Schüpbach & Baur 2008b, 2010b; Wacker 2008), but the fitness costs of parasitism in limacid slugs is unknown. We tested whether slugs showed avoidance of parasitised conspecifics by determining responses to conspecific mucus trails on soil and within shelters. In the first assay, slugs showed a nonsignificant preference for the side of the arena containing conspecific mucus trails, consistent with observations of trail following in this species for the purpose of finding food and potentially also shelter and mates (Cook 1992). Significantly, however, there was no difference in responses to mucus from parasitised and unparasitised conspecifics. In the second assay, the first choice of shelter was random with respect to the infection status of the mucus donor. Since at this point slugs were not in close contact with mucus, initial shelter selection arguably may not provide a strong test of whether slugs avoid mucus from parasitised conspecifics. More informative in this regard is whether, having entered, slugs then choose to leave the shelter. Focusing on this decision, we found a weak tendency for slugs to leave shelters more often if they contained mucus from a parasitised donor, but this difference was nonsignificant. Overall, then, we failed to find strong evidence that slugs avoid mucus from parasitised conspecifics. One possible explanation for this result is parasitism by *R, oudemansi* may not impose significant fitness costs on hosts, or at least levels of cost that outweigh the benefits of mating and huddling, with the result that avoidance behaviour is not favoured by selection. As noted above, this cannot be addressed until more data are available on the costs of parasitism in this species, which would ideally include estimates of lifetime reproductive success in parasitised and unparasitised individuals. A second explanation for the absence of avoidance responses is that individuals may not be able to assess the infection status of conspecifics from exposure to their mucus. While the results of our experiments indicate that mites are able to discriminate mucus of parasitised and unparasitised slugs (see below), it is possible that this difference is not detectable to slugs.

While we generally expect hosts to minimise the risk of acquiring parasites through avoidance of infected conspecifics, behavioural manipulation by parasites can result in parasitised individuals engaging in behaviours that actively increase parasite transmission. Examples of such behavioural modifications have been reported in gastropods, with endoparasitic trematodes appearing to drive changes in habitat use by intermediate snail hosts to ensure effective dispersal of infective stages (Curtis 1987; Lowenberger and Rau 1994). In the case of *Riccardoella* mites, movement to a previously unparasitised host may increase resource availability for mites currently facing high levels of competition for host resources, while movement to another parasitised host may afford opportunities for outbreeding. If parasites were able to manipulate host behaviour, we may then expect to see attraction by parasitised hosts towards the mucus of conspecifics, with this attraction potentially mediated by their infection status. However, we found no evidence for this. Parasitised slugs did not show a stronger preference for conspecific mucus, either on the substrate or in shelters, than unparasitised slugs, nor did the strength of preference for mucus depend on the infection status of the mucus donor. Behavioural manipulation by parasites requires control of the host nervous system, which is most readily achieved when parasites live within host tissues, or when they are able to deliver neuroactive compounds or endosymbionts into hosts (Adamo 2013; Weinersmith 2019). For *Riccardoella* mites, which live on the body surface and whose reach into their host extends only as far as the pallial cavity from which they suck haemolymph, the ability to alter the functioning of the slug's nervous system may be more limited, or non-existent. While behavioural changes have been reported in snails infected with *R. limacum*, these changes, which include a reduction in feeding rate (Wacker 2008) and overall activity (Graham et al. 1996; Schüpbach & Baur 2008b), are unlikely to represent manipulation by parasites to increase transmission but may instead represent host responses to the loss of resources to parasites, as suggested by Schüpbach & Baur (2008b).

While *Riccardoella* mites spend the majority of their time on their host, they are able to walk off their host and traverse the substrate over short distances. Previous authors have suggested that transmission via the substrate may be facilitated by mucus trails (Turk and Phillips 1946; Schüpbach & Baur 2008a) but definitive evidence has been lacking. Our results support this idea: mites tended to move between hosts more frequently in the presence of continuous mucus trails, though this difference was not statistically significant. It should be noted that separation of slugs to prevent contact between mucus trails resulted in a slightly greater minimum distance of 2 cm between hosts than in trials where mucus trails could touch. However, given that transmission success was recorded after 72 h and that mites can travel over large distances very quickly (e.g. from the head to the tail of an adult slug measuring 10 cm in length in several seconds; M. J. R., R. E. A., K. E. S. & J. P. G., pers. obs.), it is unlikely that the difference in transmission success we observed is due to the small difference in minimum distance between hosts. While we found that mites tended to move between hosts more frequently in the presence of connecting mucus trails, mites were also capable of moving across bare soil to locate new hosts, potentially searching for new mucus trails. However, this experiment investigated transmission over a relatively small scale and it is possible that dispersal by mites over greater distances depends more strongly on the presence of mucus trails. Precisely why transmission between hosts is facilitated by mucus remains to be determined. One possibility is that mite locomotion is more effective on mucus: mites are presumably adapted for walking on the mucus-coated surface of the slug's body and as a consequence may be less efficient at traversing other kinds of substrate, though anecdotally we did not observe a clear difference in the speed of movement between mites on hosts versus soil (M. J. R., R. E. A., K. E. S. & J. P. G., pers. obs.). Alternatively, or additionally, the presence of mucus trails may help mites to locate new hosts more rapidly, minimising the time spent off hosts.

If mucus facilitates transmission, we should expect that it is attractive to mites. Previous work by Schüpbach & Baur (2008a) revealed that mites preferred to spend time on mucus versus an inert substrate but only when the mucus was fresh. However, in this experiment, the mucus was taken from the mite's own host, meaning that a preference for mucus might simply reflect an attraction to cues from a mite's own host, potentially encouraging continued contact between mite and host. In our study, we first presented mites with mucus from unparasitised slugs and parasitised slugs that were not host to the mites whose choices we were analysing. We found that mites show a preference for mucus from parasitised slugs, but only when fresh. This result implies that there is a difference in the mucus of parasitised and unparasitised slugs that is detectable to mites. This information is most likely conveyed by chemical cues, with the lack of preference for aged mucus pointing to a specific role for volatile compounds. Indeed, volatile organic compounds, or VOCs, have been shown to function as attractants in other host-parasite systems (Bailey et al. 2006; Dillman et al. 2012), as well as in other types of symbiotic relationships (e.g. mutualisms; Kandasamy et al. 2019). However, while mites showed a preference for cues from parasitised hosts, they did not discriminate between mucus from their own host and from a second parasitised slug. This could suggest that mites lack the ability to recognise their own host, either because there is insufficient variation in odour cues among hosts or because mites lack a mechanism for detecting differences in host odours. Alternatively, mites may be able to perform such recognition but in the context of the experimental assay recognition of their own host was unimportant. In the absence of evidence for familiarity with own host odours, a generalised attraction to parasitised slugs may be sufficient to allow mites to relocate hosts rapidly in the event that they become separated, while also facilitating movement between parasitised hosts, which creates opportunities for outbreeding. At the same time, a preference for parasitised slugs over unparasitised slugs may also allow searching mites to avoid unsuitable hosts (for example, juvenile or diseased slugs, or slugs with high levels of anti-parasite defences) in favour of their own or another parasitised host. An important step in teasing apart these scenarios will be to determine whether differences in odour cues between parasitised and unparasitised slugs reflect intrinsic differences in levels of anti-parasite defences, or whether such differences are the result of parasite-mediated changes in host odour (Difford et al. 2022).

In conclusion, our study of transmission of *R. oudemansi* between *L. maculatus* hosts confirms the existence of direct transmission during host-host contact and reveals the potential for indirect transmission across the substrate, facilitated by host mucus trails, to which mites are attracted. In contrast, transmission dynamics do not appear to depend strongly on host responses, with little evidence for either avoidance responses to parasitised conspecifics by unparasitised slugs or increased attraction to conspecifics by parasitised slugs. *Riccardoella* mites parasitise a number of slug species, including other limacid slugs (Zabludovskaya and Badanin 2010) and variation in host ecology and behaviour across species is likely to generate differences in transmission and other aspects of the host-parasite relationship. For instance, *Limaculus* species show pronounced differences in territorial behaviour, resulting in variation in space use and aggregation (Cook 1981a), which in turn is likely to have important consequences for host-host transmission and the spatial structuring of mite populations. Given the relative ease with which parasite burdens can be quantified and the behaviour of both hosts and parasites can be studied, we encourage further investigation into the interactions between *Riccardoella* mites and their slug hosts. Improved knowledge of the mechanisms of transmission of *Riccardoella* mites will be important not only for understanding the factors determining the distribution and intensity of parasitism within gastropod populations, but also for understanding the threats posed by mites to commercial snail farming (Graham 1994), as well as their potential as control agents of pestiferous gastropods (Fain 2004).

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Data Availability The datasets analysed during the current study have been deposited in the DRYAD repository (https://doi.org/10.5061/dryad.wwpzgmsnr).

Declarations

Ethics declarations No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated invertebrate species.

Consent to participate Not applicable.

Consent for publication All authors approved the submitted draft of this manuscript and agree to be responsible for their contributions to this publication.

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