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ORIGINAL ARTICLE

Eggs in faeces: defensive mechanisms and effects of faecal coating by a water scavenger beetle, *Coelostoma stultum* (Coleopterea: Hydrophilidae)

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Abstract

The egg stage in insect development is vulnerable to fluctuations in environmental conditions and attacks by natural enemies. Protective devices are effective means of avoiding both abiotic and biotic damage to eggs. Although some insects use their faeces as a protective device, few studies have focused on using faeces for egg protection, and studies that examined the mechanism are lacking. Females of a water scavenger beetle, *Coelostoma stultum*, typically lay eggs and coat them with cocoons and their faeces. The efficacy of a double defensive device, however, remains uncertain. Here, we conducted field observations and laboratory experiments to assess the protective effects of cocoons with faecal coating on eggs against predation and determine the duration and mechanisms of this defence. Our findings reveal that the faeces on the egg cocoon protected eggs from predation by the pill bugs, *Armadillidium vulgare*, and marsh slugs, *Deroceras laeve*. Laboratory experiments showed that the defensive effect of faecal coating was maintained for three days and decreased daily. The double protective traits with faecal coating on the egg cocoons protected the eggs from instense predation pressure in *C. stultum*. The behavioural patterns of the pill bugs and egg predation rates indicate that the faecal coating behaviour in *C. stultum* protects eggs with chemical compounds and textural camouflage in mud when the antennae of the pill bugs touch faeces. It is important to note that for this defence to be effective, the chemistry and texture of the faeces should be similar to that of the oviposition sites.

Keywords Anti-predator defence · Aquatic insect · Egg deposition · Oviposition behaviour · Parental care · Predator–prey interaction

Introduction

The egg stage in the life history of insects is immobile, making the egg vulnerable to changes in the environment (e.g., desiccation and extreme temperature change) and attack by natural enemies (e.g., microbial disease, predators, and parasitoids) (Hilker 1994). To increase egg survival, parental egg care has evolved, for example, parental behaviour associated with egg protection, oviposition site selection and protective devices (Hilker 1994). Parental behaviour associated with egg protection and oviposition site selection are common strategies to increase the survival rate of eggs (Williams 1981; Grossmueller and Lederhouse 1985; Tallamy and Wood 1986; Nafus and Schreiner 1988; Higashiura 1989; Machado and Oliveira 2002; Ohba and Maeda 2017; Santos et al. 2017; Woodman 2017). Another type of parental egg care is 'protective devices' (e.g., resemblances to plant seeds and parasitised eggs, disruptive colouration, and poisonous fluids) (Hinton 1981). For example, egg stalks in Chrysopidae protect against egg predation and cannibalism (Chen and Young 1941; Růžička 1997; Hayashi and Nomura 2014), and mantis females construct the ootheca to prevent desiccation of the eggs during the overwintering period (Birchard 1991). Protective devices effectively reduce abiotic and biotic damage to eggs and do not require parental attendance.

The materials used in protective devices vary among the insect species. Some herbivorous insects use compounds

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from their host plants, such as toxic compounds, wax-like strands, and scales, as an ingredient in the protective device of eggs (Peterson 1963; Hinton 1981). Additionally, some insects use their own secretions. Chrysomelidae and Curculionidae (Sherman and Tamashiro 1954; Hilker 1994) use their faeces as protective devices. Faeces are liquids or solids containing undigested food and metabolic excretions (Gullan and Cranston 2010) discharged as unusable materials. However, faeces have thixotropic properties similar to mud and chemical properties that can be used as scent markers and toxins (Stewart et al. 2001; Brown and Funk 2010). Females of the sweet potato weevil, *Cylas formicarinus* (Fabricius, 1798) (Coleoptera: Brentidae), excavate a hole in the potato to lay eggs and plug the entrance with faeces (Sherman and Tamashiro 1954). It is speculated that the faecal plug maintains moistened conditions for the egg and protects it from mechanical injury and predators (Sherman and Tamashiro 1954). Regarding the use of faeces by insects, particular attention has been placed on the faecal case and shield of chrysomelid larvae. Many studies have shown that their shields protect them from predators either physically or by chemicals derived from their host plants (Eisner et al. 1967; Olmstead and Denno 1993; Gómez et al.1999; Vencl et al. 1999). In contrast, few studies focus on using faeces for egg protection, and studies that examine the mechanism are lacking. Damman and Cappuccino (1991) clearly demonstrated that the faecal covering of egg masses in chrysomelid beetles reduced egg mortality resulting from sucking and chewing by predators and suggested that the reduction is due to physical barriers or repellents.

The water scavenger beetle *Coelostoma stultum* (Walker, 1858) (Coleoptera: Hydrophilidae) typically lays egg cocoons in hollows in wet mud or rocks (Matsushima 2019). The egg cocoons are bowl-shaped and entirely wrapped in silk. The sticky liquid silk becomes elastic as it dries, which would exhibit a degree of efficacy in protecting eggs. Additionally, the females of this species coat the central area of the lid of the cocoon with their wet faeces (Matsushima 2019). The behaviour of double-layering substrates to cover eggs, as observed in *C. stultum*, is rare among insects. The reason they cover eggs so meticulously may be due to intense predation pressure. Clarifying the function of faecal coating will shed light on the relationship between underlying external pressures and egg protection in insects. Another area of interest is the relationship between the mechanisms of egg protection using faeces and feeding habits. Unlike herbivores feeding on host plants containing noxious compounds, the genus *Coelostoma* feeds on decaying plant material and detritus (Minoshima et al. 2018). It is thought that egg protection by faeces is strongly associated with the feeding habits of parents; therefore, it may function by a different mechanism from that of other faeces-using insects. This research will also make a crucial contribution to discussing the effects of feeding habits on egg protection in insects.

In this study, we investigated the effects of faecal protection on egg survival and its causes in a field experiment. We additionally conducted two laboratory experiments to clarify the defence mechanism and duration of the effects of egg protection by faecal coating in *C. stultum*. In laboratory experiments, we used a dominant predator of *C. stultum* and investigated predation rates between egg cocoons of different ages. We then compared the predation rates and responses of predators to egg cocoons coated with different materials.

Materials and methods

Study animals

The genus *Coelostoma*, including *C. stultum*, usually inhabits the surrounding edges of different water bodies, such as rivers, paddy fields, and ponds containing decomposing organic material. They are more active at night (Jia et al. 2019; Liu et al. 2020). Hydrophilid females arrange their eggs within silken cocoons. Silk is produced by modified ovarioles and oviduct glands and is attached to the substrate or part of the cocoon (Hinton 1981; Lancaster and Downes 2013). The oviposition behaviour of *C. stultum* has been classified into seven steps: the mated female uses a slightly hollowed site in wet mud as the oviposition site or makes a hollow in the wet mud by using her head to indent for laying eggs (Fig. 1a); then, silk is produced from the genitalia and the bottom of the egg cocoon is spun (Fig. 1b); after that, several eggs are laid on the bottom of the egg cocoon, and the partitions are spun with the silk covering the eggs (Fig. 1c), then, several eggs are laid on the partition (Fig. 1d), and the lid of the egg cocoon is spun to cover all the eggs (Fig. 1e). Finally, the female coated the lid of the egg cocoon with her faeces (Fig. 1f).

Study site

Experiment 1 was conducted in six adjacent paddy fields in Tsukuba, Ibaraki Prefecture, Japan (36°06′60"N, 140°07′43"E [DMS], approximately 8 m above sea level). These fields are surrounded by other paddy fields, vegetable fields, small forests, and ditches. The ridges of the paddy fields are muddy and have sparse vegetation and dead grass.

Experiment 1: The effects of faecal coating on egg cocoons in the field

We conducted a field experiment from May 12 to June 21, 2019, to investigate whether faecal coating affected egg hatchability. We walked on the ridge of paddy fields and

Fig. 1 The oviposition behaviour of *Coelostoma stultum* (Walker, 1858). **a** the female indents the mud using her head for the oviposition site; **b** the female produces the silk and spins the bottom part of the egg cocoon; **c** the female spins the partition with silk after laying some eggs; **d** the female lays some eggs on the partition; **e** the female spins the lid part of the egg cocoon; **f** the female finally coats the lid part of egg cocoons with her faeces

found females spinning egg cocoons on wet mud near the waterside. We stuck a pin made from a bamboo skewer into the mud near the female spinning the egg cocoon and periodically observed the egg-laying behaviour. To examine the effect of the faecal coating, we prepared two treatments: no-coating and a control. After the female finished laying all the eggs and spun the lid of the egg cocoon, we removed the female to prevent her from depositing faeces on the egg cocoon (no-coating treatment, $N=20$). As a control treatment ($N=18$), we allowed the females to coat their egg cocoons with faeces. To observe the presence and behaviour of natural enemies immediately after these treatments, we visited and observed these egg cocoons approximately three to five times periodically during 30 min. These treatments and observations were conducted during clear or cloudy nights (ca. 21:00 to 0:00). After three days, we collected the egg cocoons and brought them to the laboratory. All egg cocoons were placed into separate plastic cups (7.6 cm diameter × 3.7 cm height) with a piece of moistened filter paper. Each cup was maintained at 25 °C under a 16L8D photoperiod in an incubator (LTE-1000; Tokyo Rikakikai Co., Ltd., Tokyo, Japan).

Egg cocoons in which at least one egg hatched were defined as 'hatched', and those where no egg hatched were defined as 'unhatched'. To examine whether the presence of faeces affects embryonic development and egg survival, we observed the egg cocoons daily. We recorded the incubation period before hatching, the period during which all larvae emerged from the egg cocoon, and the number of hatched larvae. The incubation period before hatching was defined as the period from which egg cocoons were laid until the first larvae hatched. To determine the cause of unhatched eggs, we observed the exterior of the unhatched egg cocoons and dissected them under a microscope using tweezers. If the cocoon had a bite mark or the lid was missing, it was defined as a preyed-upon egg cocoon. If the cause was unclear, it was recorded as such.

Experiment 2: The duration of the faecal coating for egg protection

Experiment 2 was conducted in the laboratory from June 4 to July 26, 2020, to examine the duration of the defensive effect of the faecal coating. The egg cocoons were assigned to two treatments: no-coating and the control, similar to Experiment 1. We placed an individual in a plastic cup (7.6 cm diameter × 3.7 cm height) with a piece of moistened filter paper. We defined the day of oviposition as day 1 and prepared six treatments of egg cocoons of different ages (days 1, 3, and 5) and faecal coatings (present and absent). Based on the results of Experiment 1, the mean incubation period for hatching was approximately 7 days at 25 °C. Therefore, we defined the egg cocoons on day 1 as the early period, day 3 as the middle period, and day 5 as the late period. The pill bug *A. vulgare*, the dominant predator of the egg cocoons of *C. stultum*, was used as the predator in the laboratory experiment. All experiments used adult females of the predator to eliminate behavioural differences between the sexes. Pill bugs were collected from the same field site as in Experiment 1 and brought to the laboratory. The collected pill bugs were placed into separate plastic cups (7.6 cm diameter × 3.7 cm height) containing wet mud (0.5 cm height) for two days to starve and were only used once in the experiments. Wet mud was collected from the soil surface at the study site using a shovel. The plastic cups were maintained at 25 °C under a 16L8D photoperiod in an incubator (LTE-1000; Tokyo Rikakikai, Tokyo, Japan).

The experiment was conducted under a red light in an incubator. The plastic cups with wet mud were used as the experimental arena. One egg cocoon was placed in a cup, and the bottom portion was buried in mud, as seen in the field. We placed one pill bug on the opposite side of the egg cocoon in the arena. We recorded whether the eggs had been eaten one hour later. The unbroken egg cocoons were reused twice. All treatments were replicated 20 times.

Experiment 3: The mechanisms of the faecal coating for egg protection

Experiment 3 was conducted from June 26 to July 4, 2020, to clarify the defensive mechanisms of faecal coating. Using egg cocoons and pill bugs collected in the same way as in Experiment 2, the egg cocoons on day 3 were assigned to four treatments: control ($N=20$), no-coating ($N=20$), egg cocoons with an artificial faecal coating (artificial coating, $N=21$), and mud coating (mud coating, $N=20$) (Fig. 2). In the artificial treatment, we used the fresh faeces excreted within 2 h of the collection of *C. stultum* adults and the mud from the study site, respectively. We coated the lid of egg cocoons with approximately the same amount of coating as that of the control. In the arena, we presented the egg cocoons of each treatment to a pill bug. Digital video cameras (SONY HDR-500 and SONY HDR-CX630; Sony, Tokyo, Japan) were used to record the response of pill bugs to egg cocoons in different treatments. We analysed the presence or absence of predation one hour after the predator was introduced into the arena. Unbroken egg cocoons were reused twice. We classified the behaviours of the pill bugs into five categories: (1) *approaching*, (2) *passing over*, (3) *turning back*, (4) *searching*, and (5) *preying* (for details, see Table 1 and Fig. 3). The transitions from *approaching* to *passing over* or *turning back* were considered as an indication that the pill bug was not interested or repelled. We regarded the transition from *approaching* to *searching* as an interest in and exploration of the egg cocoon for food by the pill bug. The transition from *searching* to *passing over* occurred when the pill bug recognised the egg cocoon as a non-food resource.

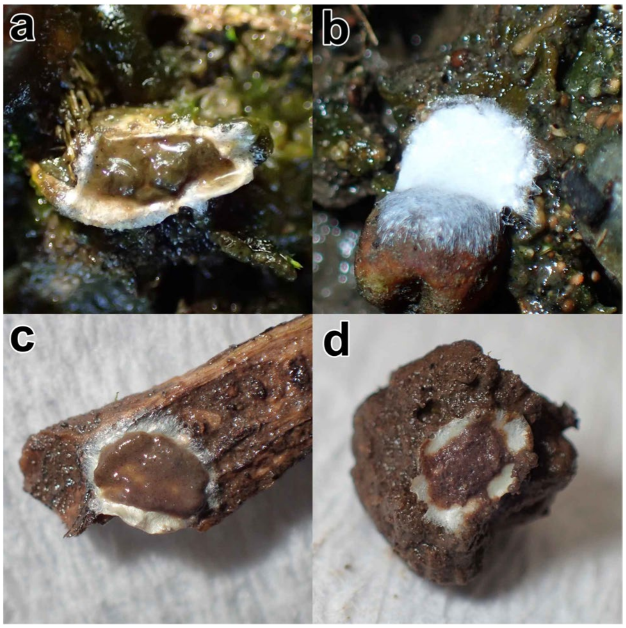

Fig. 2 *Coelostoma stultum* egg cocoons with a faecal coating (**a**), without a faecal coating (**b**), with an artificial faecal coating (**c**), and with a mud coating (**d**)

Statistical analysis

The data were analysed using statistical software R version 4.0.2 (R Core Team 2020). Fisher's exact probability test was used to compare the percentages of egg cocoon status between the control and no-coating groups in Experiment 1. The Mann–Whitney U test was used to compare the differences between treatments (control and no-coating) on the number of larvae and the incubation period of the hatched egg cocoons. For Experiment 2, a generalised linear model (GLM) with a binomial error distribution (a logit link function) was used to analyse the faecal coating's duration for egg protection. The presence of predation was used as a response variable, which was non-over-dispersed binary data. The proposed GLM consisted of the following explanatory variables; the presence of faecal coating, the day after oviposition (days 1, 3, and 5) and the interaction between them. To consider the effect of the interaction between explanatory variables, we used AIC to select the best-fitting model. We considered models with ΔAIC (difference in the AIC value from the best model) <2.0 as plausible models. Fisher's exact probability test was used to compare the predation rates of egg cocoons of

Table 1 Ethogram of observed behaviours in *Armadillidium vulgare* (see Fig. 3)

Behaviour	Definition
Approaching	The pill bug moves towards the egg cocoon and touches it
Passing over	The pill bug walks on or beside the egg cocoon at an angle more than 90 degrees from the angle of incidence
Turning back	The approached pill bug pulls back from the egg cocoon at an angle less than 90 degrees from the angle of incidence
Searching	The approached pill bug stays on or near the egg cocoon and touches it with its antennae. Sometimes the pill bug gnaws on the surface of the egg cocoon
Preying	After searching, the pill bug preys on the eggs inside the egg cocoons

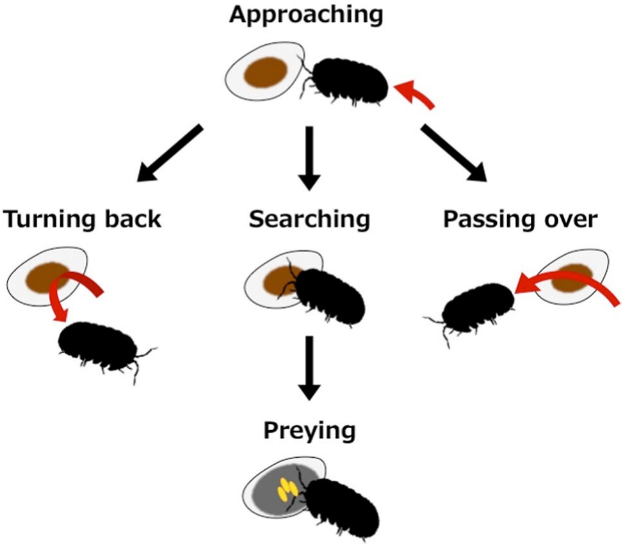

Fig. 3 Behavioural pattern in the pill bug, *Armadillidium vulgare*. Black arrows indicate the transition of behavioural elements, and red arrows indicate the trajectory of the pill bugs

different materials in Experiment 3. Eight recordings in which predator behaviour could be clearly observed were selected from each treatment and analysed by first-order Markov chain analysis (in the 'markovchain' package in R (Spedicato 2017)). This analysis calculates the likelihood of all transitions between behavioural patterns (Slater 1981). To compare the effects of treatment on the behavioural patterns of pill bugs, we conducted multiple comparisons between treatments using Fisher's exact probability test. Holm's correction was used for post hoc analysis. *P*-values less than 0.05 were considered statistically significant for all tests.

Results

Experiment 1

The ratio of hatched egg cocoons in the no-coating treatment was significantly lower than that of the control (no-coating treatment: 40.0%, control: 83.3%; Fisher's exact probability test, $P < 0.01$, Fig. 4). In the no-coating treatment, the lids of many of the unhatched egg cocoons were removed by pill bugs, *Armadillidium vulgare* (Latreille, 1804) (Isopoda: Armadilloidea) (40.0%), and some had bite marks from the marsh slug, *Deroceras laeve* (Müller, 1774) (Gastropoda: Agriolimacidae) (10.0%) (Fig. 4). The cause of 10.0% of unhatched egg cocoons is unknown. No parasites emerged from any egg cocoons. In the field, pill bugs and marsh slugs approached and preyed on all the eggs inside the egg cocoons without coating (Fig. 5a, b). Occasionally, these predators started to prey within a few minutes after the treatment was set. They bit and broke the lids of egg cocoons and preyed on the eggs. In cases where pill bugs consumed the eggs, the lid completely disappeared from the cocoon, whereas *D. laeve* created one or a few holes in the lid (Fig. 5c, d).

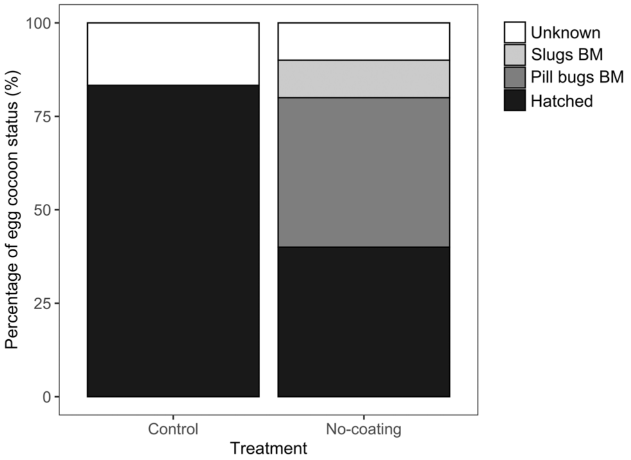

Fig. 4 The percentage of egg cocoons status between control and no-coating in *Coelostoma stultum*. 50% of the no-coating egg cocoons had bite marks (BM) by pill bugs, *Armadillidium vulgare*, or marsh slugs, *Deroceras laeve* (hatchability, control: 83.3%; no-coating: 40.0%, Fisher's exact probability test, $P < 0.01$)

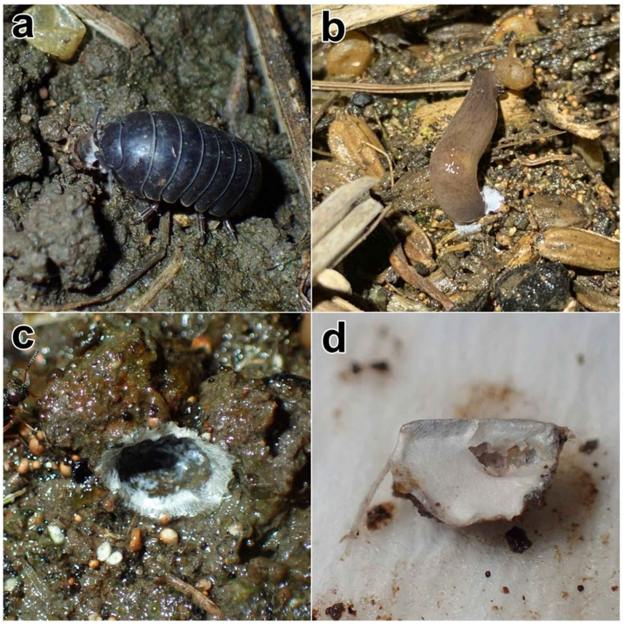

Fig. 5 Predation events and bite marks on the egg cocoons of *Coelostoma stultum* by the pill bug, *Armadillidium vulgare*, (**a**, **c**) and the marsh slug, *Deroceras laeve* (**b**, **d**)

Hatchability of egg cocoons without bite marks was not significantly different between the control ($N = 18$) and no-coating ($N = 10$) (Fisher's exact probability test, $P = 1.00$). In the hatched egg cocoons (control: $N = 15$; no coating: $N = 8$), the incubation period before hatching and the number of hatched larvae were 7.4 ± 0.6 days (mean ± S.D.) and 11.7 ± 3.9, respectively. There were no differences between treatments (Mann–Whitney U test, incubation period: $U = 71$, $P = 0.44$; number of hatched larvae: $U = 67.5$, $P = 0.65$, Fig. 6).

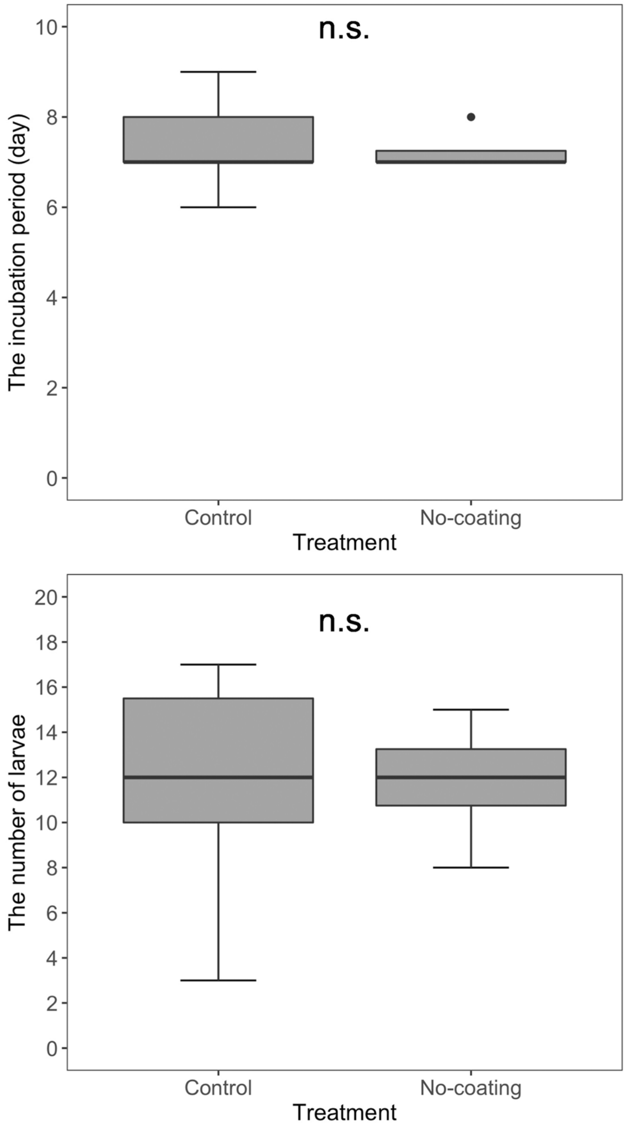

Fig. 6 **a** The incubation periods of the hatched egg cocoons for the control and no-coating egg cocoons in *Coelostoma stultum*. **b** The incubation periods of the hatched egg cocoons for the control and no-coating egg cocoons in *Coelostoma stultum*

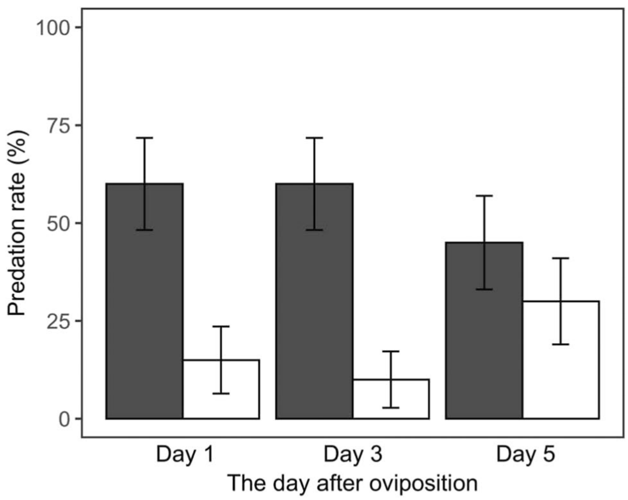

Fig. 7 Temporal changes in the effects of the faecal coating on the predation by the pill bug, *Armadillidium vulgare*. Closed and open bars indicate no-coating and control, respectively. Error bars indicate a 95% confidence interval

Experiment 2

The predation rate of egg cocoons for the no-coating treatment was higher than that for the control for all treatments (Fig. 7). In the predation rate models (Table 2), the best model contained only the presence of faecal coating. The next best-supported model incorporated an additional effect of the interaction between the presence of faecal coating and the day after oviposition. Third, the full model was supported. In the control treatment, 95% confidence intervals of the mean predation rate did not overlap between days 3 and 5, i.e., the predation rate of the egg cocoons in the control on day 5 was significantly higher than that on day 3 (Fig. 7).

Table 2 Results of AIC model selection for the effects of the presence of faecal coating and the day after oviposition on the predation rate on *Coelostoma stultum* eggs by the pill bug, *Armdillidium vulgare*

Model	AIC	ΔAIC	Deviance
fc[1]	143.75	0.00	137.31
fc + fc:day[2]	144.22	0.47	138.22
fc + day + fc:day	145.31	1.56	137.31
fc + day	145.75	2.00	139.75
day + fc:day	154.47	10.73	148.48
null	159.72	15.97	157.71
day	161.72	17.97	157.71

Models with ΔAIC (difference in the AIC value from the best model) < 2.0 are considered plausible

[1] fc, the presence of faecal coating; [2] day, the day after oviposition

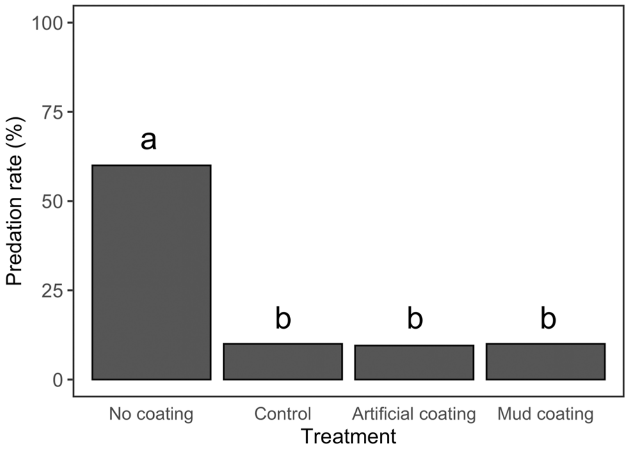

Fig. 8 The predation rate of *Coelostoma stultum* egg cocoons by the pill bug, *Armadillidium vulgare* for each treatment. The different letters above the bars indicate significant differences using Fisher's exact probability test, $P < 0.05$, adjusted by Holm's correction

Experiment 3

The predation rates of the egg cocoons with artificial and mud coatings were similar to that of the control. The rates for the control, artificial coating, and mud coating were 10.0%, 9.5%, and 10.0%, respectively (Fig. 8). Predation rates in these three treatments were significantly lower than no-coating treatment, which has a rate of 60.0% (Fisher's exact probability test with Holm's method correction, $P < 0.01$, Fig. 8).

Markov chain diagrams for each treatment are shown in Fig. 9. In all four treatments, the transition from *approaching* to *passing over* had a high likelihood (62.5–79.3%), whereas that from *approaching* to *turning back* was 1.9–10.8% (Table S1, Fig. 9). Except for the no-coating treatment, the likelihood of the transition from *searching* to *passing over* was higher than to *preying* (94.7–96.0%). In egg cocoons without coating, the likelihood of the transition from *searching* to *preying* tended to be higher than in the control (Fisher's exact probability test with Holm's method correction, $P = 0.06$).

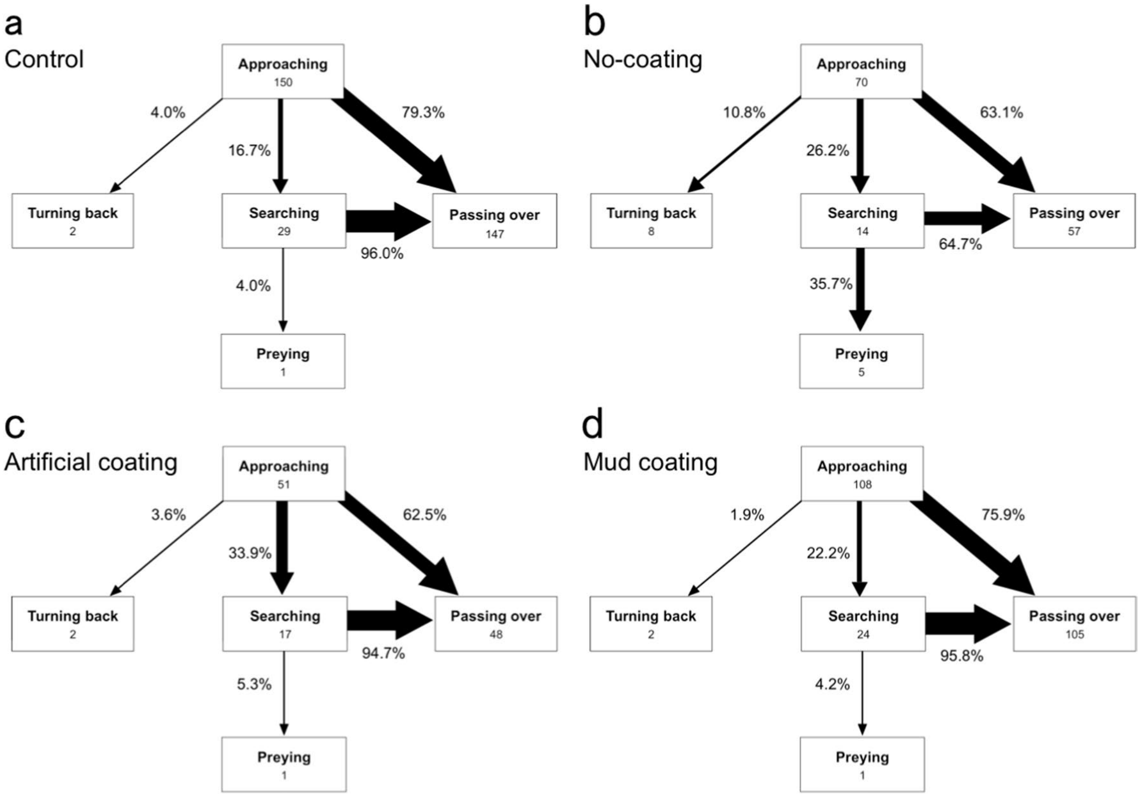

Fig. 9 The Markov chain diagrams for each treatment. The numbers under the behavioural elements indicate the number of times the behaviour was observed in eight predators for each treatment. In this analysis, the individual difference was not considered. The percentages near each arrow indicate the transition probability and are reflected in the arrow's thickness. Egg cocoons of control (**a**), no-coating (**b**), with an artificial faecal coating (**c**), and with a mud coating (**d**)

Discussion

The effects of faecal coating on egg cocoons in the field

The use of faecal materials for egg protection in insects has been discussed as a protection against solar radiation, desiccation, predators, and parasitoids (Chaboo et al. 2007; Hilker 1992; Olmstead and Denno 1992; Olmstead 1994). This study showed that the faeces coating egg cocoons in *C. stultum* protect against predators such as the pill bug, *A. vulgare*, and marsh slug, *D. laeve*. The predation rate of egg cocoons in the no-coating treatment was higher than that of the control. This suggests that the faecal coating behaviour of *C. stultum* females plays an important role in egg protection against these predators. Based on Experiment 1, 40% of the no-coating egg cocoons were preyed upon by pill bugs, which are considered the most dangerous egg predators for *C. stultum*. In fact, the pill bugs approached the cocoon and started feeding within ten minutes of the no-coating treatment in the field. In the hatched egg cocoons, the number of larvae and the incubation periods were similar between the egg cocoons with and without faecal coating, indicating that faecal coating has no positive or negative effect on embryonic development.

Notably, the faecal coating in *C. stultum* was not applied directly to the eggs but to the egg cocoons that enfold the eggs. Their protection style is different from egg protection in Chrysomelidae and Curculionidae, where eggs are directly covered or plugged with faeces (Chaboo et al. 2007; Damman and Cappuccino 1991; Sherman and Tamashiro 1954). Since the egg cocoon is one of the traits for egg protection (Hinton 1981), the egg cocoons themselves may also help maintain humidity and protect the eggs from other natural enemies, such as ants, ground-dwelling beetles, and fungus. However, our results showed that egg cocoons alone could not avoid predation pressure from pill bugs and slugs. The double protective trait with faecal coating on the egg cocoons effectively protectedthe eggs from them in *C. stultum*.

The duration and mechanisms of egg protection by faecal coating

The presence of faecal coating decreased the predation rate by pill bugs in the laboratory. Our results suggest that the defensive effect of faecal coating persisted until day 3, the middle period of the hatching timeframe. Although the predation rate on the egg cocoons in the control was consistently lower than that in no-coating, it was significantly higher on day 5 than on 3. This reduction in defensive effects on day 5 may be due to the deterioration of faeces; the drying over time may be causing the faeces to crack and become fragile. In addition, there was a tendency for egg cocoons without coating to be less prone to predation on day 5 than on days 1 and 3, implying that there was no longer a difference between treatments. This may be explained by the reduction of odours released from egg cocoons, which predators use as a cue to recognise food.

Previous studies have suggested that the protective devices using faecal materials function as chemical repellent barriers (Morton and Vencl 1998; Müller and Hilker 1999), physical barriers (Eisner et al. 1967; Nogueira-de-Sá and Trigo 2005), and visual camouflage (Chaboo et al. 2007). The predation rates of egg cocoons with faecal, artificial, and mud coatings were lower than that of no-coating (Fig. 8). Furthermore, behavioural analysis of the pill bug predators showed that the behavioural patterns of the artificially coated and mud-coated egg cocoons were similar to those of the control treatment (Fig. 9). These results suggest that the faeces coating the egg cocoons do not include unique compounds such as repellent chemicals produced by females, but that faeces have similar properties to the faeces that adults usually excrete and even the detritus-laden mud they feed on. The faeces or mud on the egg cocoon may prevent the pill bug from recognising the cocoon after being touched by the antennae. The likelihood of transition from *searching* to *preying* tended to be higher in egg cocoons without faecal coating than in the controls. Pill bugs have poorly developed eyes and rely on olfactory cues by using their antennae when searching for food (Iwata and Watanabe 1957), while they have strong mandibles that can chew on hard substrates, including solid leaves (Schmitz 1986). We observed that the pill bugs gnawed and sometimes fed on faeces or mud only, leaving the egg cocoon. Therefore, it was suggested that the defence mechanism of faecal coating for egg protection in *C. stultum* is not a chemical repellent or physical barrier but a chemical or textural camouflage in mud, which acts when the antennae of pill bugs touch faeces.

Several studies have indicated a trade-off between the degree of protection and survival. In the leaf-mining hispine beetle *Microrhopala vittata*, females cover each stack of eggs with brown liquid excreta, called faecal cover (Messina and Root 1980). This structure interferes with predators, including erythraeid mites and ants, while it attracts the eulophid wasp, resulting in higher mortality due to the parasite (Damman and Cappuccino 1991). Brown and Funk (2005) suggested a trade-off between the hardness of pupal faecal cases and emerging adults. In contrast, no parasites emerged from any of the egg cocoons, and no negative effects of faecal coating on egg development or hatchability were detected in this study. The defence mechanism of chemical compounds or textural camouflage in mud in *C. stultum* does not provide any cues to enemies. Therefore, egg protection by mud camouflage may not result in a trade-off between protection and survival and may be an effective method of egg protection against various natural enemies.

Egg protection behaviour using faecal material is strongly associated with feeding habits and oviposition sites. Chrysomelidae and Curculionidae can protect the eggs with deterrent or repellent compounds that are the defensive traits of plants, suggesting that they have acquired such systems by overcoming plant-derived noxious chemicals (Borg-Karlson et al. 2006; Eisner et al. 1967; Gómez et al. 1999; Olmstead and Denno 1993; Vencl et al. 1999). In contrast, *C. stultum* feeds on detritus, which does not contain noxious materials that can be used for defence. The present study implies that the faecal coating on egg cocoons in *C. stultum* protects eggs through the similarities between the chemistry and texture of the faeces and those of the oviposition sites.

Evolution of faecal coating behaviour in *C. stultum*

The main predator of *C. stultum* eggs is the pill bug *A. vulgare*, a worldwide exotic species from Europe and widely distributed in Japan (Nunomura 1990; Schmalfuss 2003). The marsh slug *D. laeve* is another predator of *C. stultum* eggs. The native distribution range of *D. laeve* is generally interpreted to be Palearctic or Holarctic between the subpolar and subtropical zones (CABI 2021; Wiktor and Auffenburg 2002; Wiktor 2000), although it has been introduced to and found in widespread areas, including Japan (Hammond 1996; Kurozumi 2000; South 1992). In particular, the population of *D. laeve* at the study sites was shown to be an introduced population (Matsushima and Haga 2021). Meanwhile, the genus *Coelostoma* is distributed only in the eastern hemisphere, and *C. stultum* is naturally widespread in tropical to warm-temperate areas (Hansen 1999). Since the distribution of *C. stultum* did not initially overlap with that of *A. vulgare* and *D. laeve*, the faecal coating behaviour for egg protection may have been selected by the predation pressure of other native predator species. Additionally, the first author observed in the laboratory that two closely related species of the genus *Coelostoma*, *C. orbiculare* and *C. fallaciosum*, have the same faecal coating behaviour on egg cocoons. Similar behaviour has also been described as a faecal drop in the genus *Cercyon* belonging to the same subfamily as *Coelostoma*, Sphaeridiinae (Schulte 1988). These facts imply that the use of faecal material has been acquired in the ancestral group of *C. stultum* and may have been maintained over time.

In conclusion, the double protective traits with faecal coating on the egg cocoons protected the eggs from intense predation pressure in *C. stultum*. For pill bugs, which identify their food by the antennae, the faeces on the egg cocoon would act as camouflage to prevent them from recognizing it as food. The defence mechanisms would be chemical compounds or textural camouflage in mud. It is important to note that for this defence to be effective, the chemistry and texture of the faeces should be be similar to that of the oviposition sites.

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Data availability The data supporting the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval statement This study was conducted according to the principles of the Declaration of Helsinki.

Competing interests There are no conflicts of interest to declare.

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