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Research

Environmental conditions and seed traits affect seed dispersal patterns in a slug–legume model system

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Oikos
00: 1–12, 2021
doi: 10.1111/oik.08318

Subject Editor: Bente J. Graae
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 14 June 2021

The outcome of seed fate (probability of predation or dispersal) depends on the interplay between granivores and seed traits. Furthermore, changing environmental conditions affecting granivore behavior might additionally alter seed dispersal patterns.

Based on microcosm experiments and time-lapse videos, we tested in a structural equation model how dry or wet conditions in a slug–legume seed system affect slug movement behavior, thus influencing seed encounter, ingestion and dispersal locations. We additionally analyzed how interactions between slug weight, chemical and morphological seed traits determine the fate of ingested seeds and used point pattern analysis of seed locations to detect potential dispersal patterns.

While dry or wet conditions had no direct effect on the time slugs spent moving, dry conditions significantly increased the slug's homing behavior. Higher slug movement increased the chance of seed encounter, smaller seeds with low seed coat permeability were preferably ingested and the proportion of ingested seeds increased with increasing seed oil content. A high proportion of ingested seeds was dispersed via endozoochory, but none of the tested seed traits could explain seed fate.

Endozoochory led to clumped dispersal patterns around the slug's shelter with clustering significantly more pronounced for seeds in dry conditions due to increased homing behavior in slugs. These dispersal patterns indicate potential directed dispersal to moist microsites but whether this leads to higher recruitment and hence successful directed dispersal remains to be tested.

Keywords: Arion, directed dispersal, endozoochory, exozoochory, legume seeds, seed fate

Introduction

Seed dispersal and post-dispersal seed predation are biotic interactions with major implications for plant population dynamics (Lortie et al. 2004, Larios et al. 2017). They affect the number and species composition of available seeds and their location in microsites within the community and hence plant recruitment (Schupp and Fuentes 1995, Hulme 1998, Crawley 2000). Animals that interact with seeds are either obligatory or facultative granivores and may act as seed disperser, predator or fulfill both roles

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simultaneously, with opposing consequences for the seed (Retana et al. 2004). Some invertebrate groups such as carabids, earthworms, woodlice or slugs are described as facultative granivores and their interactions with seeds often result in seed predation (Hulme and Benkman 2002, Lundgren and Harwood 2012). Endozoochory (seed dispersal via ingestion) in invertebrates is seen as rare due to size limitations but has been noted in recent years for various taxa (Duthie et al. 2006, de Vega et al. 2011, Forey et al. 2011, Suetsugu 2018). For example, earthworms and slugs have been observed to be prolific endozoochorous seed dispersers for a number of seed species (Eisenhauer et al. 2009, Türke et al. 2010, Pufal and Klein 2013, Clause et al. 2017).

The extent of many invertebrate–seed interactions is often species-specific for both invertebrate and/or seed species (whether and how many seeds are ingested initially) (Honek et al. 2007, Eisenhauer et al. 2009, Clause et al. 2011, Hana et al. 2020). However, this species-specificity can also be linked to animal and seed traits that affect not only the initial interaction of seed ingestion but also the outcome of seed fate, i.e. whether ingested seeds are digested (seed predation) or excreted intact (endozoochory). Seed traits that are known to affect their palatability to granivores are seed size, seed coat hardness, seed density and nutritional quality (Kulkarni et al. 2015, Clause et al. 2017, Hana et al. 2020). In general, the size of preferred seeds correlates positively with the animal's size, as has been shown for carabids (Honek et al. 2007, Kulkarni et al. 2015), slugs (Türke and Weisser 2013), some ant species (Arnan et al. 2010) and millipedes (Koprdová et al. 2010). When seeds are ingested, they are not necessarily destroyed initially through mastication, but may arrive in the gut intact (Calvino-Cancela and Rubido-Bará 2012). How those seeds then survive gut passage and are dispersed via excretion is not well studied for invertebrates. However, research on seed ingestion and digestion by earthworms showed that seed size as well as seed oil content are seed traits that affect the digestion and hence seed fate of ingested seeds in *Lumbricus terrestris*, with smaller and fattier seeds more likely to be digested (Clause et al. 2011, 2017). The negative effect of seed size on seed digestion for *L. terrestris* was confirmed for other seed species in a more recent study (Clause et al. 2017).

Slugs are also increasingly being recognized not only as seed predators (Kollmann and Bassin 2001, Miczajka et al. 2019), but also important endozoochorous seed dispersers (Türke et al. 2010, Calvino-Cancela and Rubido-Bará 2012, Pufal and Klein 2013). Seed ingestion, predation and endozoochory can be affected by seed as well as slug traits. There is a positive link between slug size and seed size in the ingestion of seeds (Türke et al. 2010, Calvino-Cancela and Rubido-Bará 2012, Türke and Weisser 2013) and seed size also seems to be important for the proportion of seeds that are defecated undamaged, i.e. dispersed via endozoochory (Türke and Weisser 2013). Some studies addressed potential dispersal distances for seeds dispersed via endozoochory by slugs. Türke et al. (2010) calculated potential maximum dispersal distances of around 15 m by calculating maximum distances for gut retention times and Calvino-Cancela and Rubido-Bará (2012) assumed that dispersal distances would not exceed a few meters. However, how other aspects of slug movement and foraging behavior might affect slug–seed interactions has not yet been studied. Slug activity is strongly driven by ambient temperature and soil moisture (Willis et al. 2006). Speiser and Hochstrasser (1998) showed that with higher soil moisture, slugs were more active, resulting in higher herbivory levels and Nicolai and Ansart (2017) state that drought and higher temperatures might decrease slug activity. Various gastropod species including terrestrial snails and slugs show homing behavior, where they return to a microsite with favorable conditions (shelter) (Edelstam and Palmer 1950, Gelperin 1974, Rollo and Wellington 1981, Ng et al. 2013). This homing behavior increases under adverse environmental conditions, such as increasing temperatures and evapotranspiration (Rollo and Wellington 1981, Prior 1985). Based on this knowledge, we have the following hypotheses (Fig. 1a):

- Environmental conditions (i.e. surface moisture) will affect slug activity and behavior (movement and resting periods) but less so for heavier slugs.
- Slug activity and behavior patterns determine whether they encounter seeds and seed encounter probability will hence change in response to different environmental conditions.
- When a seed source is encountered, slug weight and seed traits will affect if and how many seeds are ingested.
- Whether ingested seeds are digested or dispersed will depend on a combination of different seed traits.
- Slug activity and behavior will then affect where and how far excreted seeds are dispersed.

We tested these assumptions in a microcosm experiment with slugs and legume seeds in dry and wet surface conditions.

Material and methods

Study species and traits

We used six *Trifolium* and three *Medicago* species (both Fabaceae) primarily based on their differing seed size. To analyze effects of seed traits on seed consumption, predation and dispersal, we considered the following traits for analyses: seed length and width, seed volume, seed dry mass (g/1000 seeds), seed coat permeability (SCP), seed coat thickness (SCT), oil content and protein content. These seed traits were available, and hence used, for most species from the TRY database (Kattge et al. 2011). For *T. repens* and *M.* x *varia*, which did not have all traits listed in the TRY database, we weighted ten batches of 1000 seeds in the laboratory with an ALC-210.4 analytical balance (Acculab) and used the mean as seed dry weight. We further measured seed length, width and thickness and calculated the volume for 20 *M.* x *varia* seeds and used their mean in seed trait analyses. Oil and protein content for *M.* x *varia* were provided

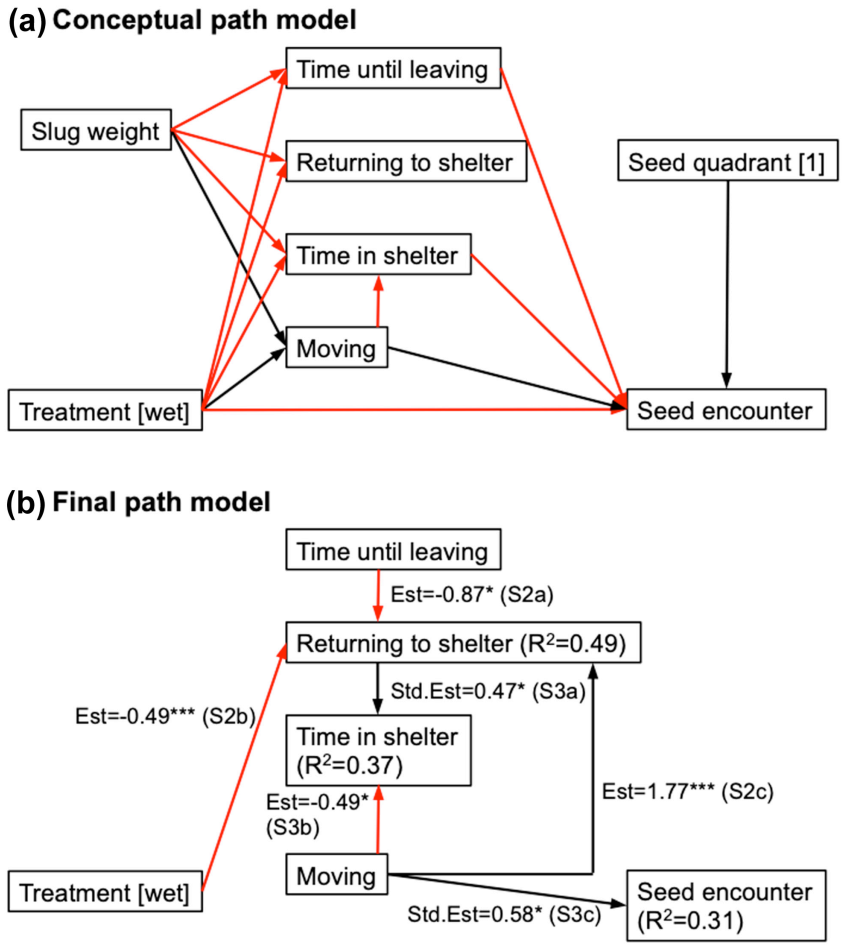

Figure 1. Conceptual path model for the relationships between treatment, slug weight, movement and seed encounter (a) and final path model for slug movement and seed encounter with predictions for each significant path (b). Shown are estimates or standardized estimates for each path with significant levels as $^{*}p < 0.05$ and $^{***}p < 0.001$. The R^2 for each model is given as Nagelkerke R^2. Positive relationships are shown with black arrows, negative relationships with red arrows. The references in brackets behind the estimates refer to the respective graphical displays of predictions in the Supporting information.

from LTZ Augustenberg (Karlsruhe, Germany) using the modified Commission Regulation (EC) 152 (2009) and MB III 4.1.2 (VDLUFA 2004) procedures, respectively. Seed coat permeability was calculated as the weight increase after water uptake. For each seed species, ten batches of 100 seeds were weighed, placed in an Eppendorf tube and immersed in 2 ml of distilled water at room temperature for 24 h (modified from Traveset et al. 2008) and then weighed again (using the ALC-210.4 analytical balance). Seed coat permeability was calculated as the percentage increase from initial to hydrated weight for each batch and the mean of all batches per species was used in the analyses. To measure seed coat thickness, ten seeds of each species were stored in 50% ethanol at 4°C for one week and then cut in half with a razor (Soons et al. 2008). Seed coat thickness was measured at five random positions on each seed half, using a Leica M165C with the Leica Application Suite ver. 4.13.0, resulting in ten measurements per seed and subsequently 100 measurements per species. The mean of these measurements was then taken as SCT for each species.

Slug individuals were collected in gardens of university employees over the course of the experiment. They were not identified to species level but were all of the genus *Arion*. Prior to the experiment, slugs were starved for 24 h and weighed just before their trial. In between trials, slugs were kept in two fauna boxes with lettuce, apple pieces and wet paper towels. If seeds were consumed and slugs did not defecate during the trial, they were kept in a separate plastic container and provided with lettuce until defecation. Due to dry weather conditions in the summer of 2017, not as many slugs could be collected and some slug individuals were used in multiple trials.

Experimental set-up

The experiment was carried out in a climate-controlled laboratory under ambient conditions in ten microcosms. The microcosms were cages with a wooden frame and mesh walls (mesh size < 1 mm) (dimensions 40 × 40 × 50 cm) with a time lapse camera affixed on top of the cage. In the bottom of the cage was a cotton dishtowel, which was either kept dry or wet to represent different environmental conditions. As shelter we provided an overturned plastic dish with two holes to allow the slugs to enter and exit and also to provide moisture continuously through a wick connected to a water bottle outside the cage. At the start of each trial, one slug was placed in the shelter in each cage and 20 seeds of one species were placed in one of four quadrants in the cage. Seeds were placed on a small plastic sheet to prevent moisture in the wet treatment from hydrating the seeds. For each cage, the slug individuals, seed species, treatment and position of seeds were assigned randomly, and for each trial run, we had five dry treatments and five wet treatments. Each trial took 48 h with a picture taken every two minutes to later analyze slug movement. After each trial, the number of remaining seeds at the original position and seeds found in slime trails were counted. We also counted the number of seeds in feces in the cage and noted their position as x-y coordinates, with the middle of the cage identified $x=0$, $y=0$. If seeds were missing, slugs were placed in a separate container until defecation and seeds in the feces were then counted. If seeds were still missing after defecation, they were recorded as digested (predated). Seeds were therefore classified as either remaining, dispersed by exozoochory (found in a slug slime trail), dispersed by endozoochory (found intact in slug feces either in the cage or in slug feces after the trial) or predated (ingested and then either found destroyed in feces or missing completely).

Video analysis

The time lapse videos were analyzed with the open software Tracker (Open source physics, <https://physlets.org/tracker>, ver. 4.4.11.0) and Elmedia Video player (<www.elmedia-video-player.com>, ver. 7.10). Due to camera battery problems, videos were not always exactly 48 h long, so we therefore noted the exact run time of each video (total time) and then took movement and resting times of the slug as proportions of the total time. In the Elmedia video app, we recorded the time when the slug left the shelter for the first time (time until leaving). We calculated the time until leaving the shelter as a proportion of the total time. The remaining time until the end of the trial was then identified as activity time. In the activity time, we recorded the duration of slug movement and whenever it stopped, for how long it rested. This gave us the total time the slug spent moving and the total time it spent resting. Movement time was calculated as a proportion of total movement from activity time. We recorded the number of times that slugs returned to their shelter (homing behavior) and how much time they spent in the shelter. Time spent in shelter was also calculated as a proportion of activity time.

In each video, we recorded whether slugs encountered the seeds. In the Tracker app, we tracked slugs frame-by-frame manually since they continuously change their shape during movement. In the app, distances can only be measured in two-dimensional space. If the slug moved in three dimensions (up-and-down the walls of the cage), these distances were estimated from a grid on the walls of the cage and then added manually to the total distance. In those trials where slugs ingested seeds at least once and defecated in the cage, we calculated the potential maximum distance of endozoochorous seed dispersal by calculating the distance from the time of seed ingestion until defecation and noted the time it took from seed ingestion to defecation (gut retention time). These data are not available for all trials where slugs ingested seeds since a) they did not always defecate during the trial, b) it was not always visible when they defecated due to either poor lighting conditions or video quality and c) when they encountered seeds more than once it was not always clear if and how many seeds they ingested at each encounter. Potential maximum dispersal distances and gut retention time can therefore only be used descriptively for the small number of available data.

Germination trials

To assess the effect of endozoochory on germination success, we compared germination rates from seeds found in feces with seeds found in slug slime and remaining in the quadrant they were originally placed in. Here, we use germination rates to describe the proportion of germinated seeds from the total number of available seeds (in either feces, slime or remaining). Remaining seeds were used as control and further supplemented with germination tests of ten seeds of each seed species in four replications. Seeds were kept on moist paper towels in small plastic petri dishes in ambient conditions. The first twenty samples were kept in those conditions for up to two weeks but no germination could be observed in any sample after five days. We hence ran all subsequent germination trials for five days for each sample.

Data analysis

Slug movement and seed encounter

All analyses were carried out in R ver. 4.0.2 (<www.r-project.org>). Slug movement and seed encounter analysis are based on the analysis of available video material. Trials were omitted from the analysis when the camera did not record or the video file was corrupted, the video quality or light conditions were not sufficient to observe movement patterns, slugs spent too much time out of frame or slugs escaped or died during the trial. This resulted in an initially usable sample of 163 out of 170 available trials. However, cameras did not always record for the entire duration of the trial and we hence only included trials were video recordings were longer than 2700 min (45 hours) since observed movement patterns could then confidently be linked to observed seed encounters. This yielded 67 usable trials for the analysis of slug movement. In two of those trials, slugs never left their shelter and we could hence not collect any movement-related variables. We omitted these trials as well, resulting in 65 trials for the movement analysis.

In a structural equation model (SEM), we tested how 1) environmental conditions and slug weight affect different movement aspects of slugs and 2) how slug movement affected the chance of encountering seeds. Our conceptual path model (Fig. 1a) assumes that slugs in the wet treatment and heavier slugs leave the shelter earlier, move more, return to the shelter less often and spend less time in the shelter. The path model also assumes that the earlier slugs leave the shelter, the more they move and the less time they spend in the shelter, increasing the chance of seed encounters. Since we placed seeds randomly in four quadrants in the microcosm, we assume that seeds in the quadrant where the shelter is have a higher chance of encounter. Based on these assumptions, we incorporated generalized linear models (GLM) into the SEM, where time until leaving the shelter, movement during the trial and time spent in shelter were proportions of the total time and hence had a binomial distribution. The response 'returning to shelter' used a Poisson distribution and seed encounter had a binomial distribution with a yes/no response. The treatment (dry/wet) was coded as binary ($1 = \text{wet}$; $0 = \text{dry}$) to simplify the interpretation of the model output of the SEM. After running the initial a priori conceptual model, the test of separation suggested further significant interactions, which we added to the respective GLMs to improve model fit (Table 1). The packages lme4 (Bates et al. 2015) and piecewiseSEM (Lefcheck 2016) were used for these analyses.

Slug–seed interactions

Prior to analyses of interactions between slugs and seeds, we tested for correlations between morphological and chemical seed traits. There were several strong correlations between different morphological seed traits (Supporting information) but not with oil and protein content or seed coat permeability. We therefore used seed coat permeability, oil and protein content as well as seed weight as a proxy for all size-related traits in models with seed traits. In all analyses of slug–seed interactions, we only used trials in which slugs encountered seeds and excluded two trials were no data on final seed fate were available and five trials were slugs either escaped or died during the trial.

To analyze interactions between slugs and seeds and their effect on seed dispersal and predation, we used a series of different response variables in our analyses (Table 1). Seed ingestion is tested in two models; firstly as a yes/no response and secondly as the proportion of ingested to provided seeds. Once seeds were ingested, they could either be digested (seed predation) or defecated intact (endozoochorous seed dispersal). Seed predation is given as the ratio of digested seeds to ingested seeds and endozoochory as the ratio of intact defecated seeds to ingested seeds. For ingested seeds, we further calculated the position for each plant species along a continuum of negative interactions (seed predation) to positive interactions (endozoochory) with the proportion of dispersed seeds (endozoochory) minus the proportion of digested seeds (seed predation). This net outcome ranged from -1 to 1 and was transformed to fit a binomial distribution $(x - \min(x)/\max(x) - \min(x))$. Seeds were also dispersed via exozoochory, where seeds stuck to the slugs' body and detached at some point during the trial. Here, we used the proportion of seeds found in slug slime trails of the total number of seeds as response variable. For all response variables, we first used a series of generalized linear mixed effect models (GLMMs) with seed species as a random effect. However, the variance of the random effect approached zero, leading to singular fit in some of the models and we therefore simply used GLMs in all analyses (Table 1). Whenever used, the fixed effects slug weight, seed weight, seed coat permeability, oil content and protein content were standardized.

Effects of the seed source (slug feces, slug slime, control) and the previously used seed traits on the proportion of

Table 1. List of models with response variable, fixed effects, error distribution (Err.Dist.) and number of trials (n). For the SEM on slug movement and seed encounter, fixed effects are hypothesized paths from the a priori path model with variables in italics as additional paths suggested by tests of direct separation. Except for exozoochory, all models for slug–seed interactions include the same fixed effects. Models on seed ingestion and exozoochory only include trials where slugs encountered seeds, whereas models concerning seed predation and endozoochory only include trials where seeds were consumed.

Response	Fixed effects	Err.Dist.	n
Slug movement and seed encounter (SEM)			
Time until leaving	Treatment, slug weight	Binomial	65
Movement	Treatment, slug weight	Binomial	65
Returning to shelter	Treatment, slug weight, *movement, time until leaving*	Poisson	65
Time in shelter	Treatment, slug weight, movement, *time until leaving*	Binomial	65
Seed encounter	Treatment, slug weight, time until leaving, movement, time in shelter, seed quadrant	Binomial	65
Slug–seed interaction			
Seed ingestion (yes/no)	Treatment, slug weight, seed weight, seed coat permeability (SCP), protein content, oil content	Binomial	128
Seed ingestion (proportion)		Binomial	128
Continuum predation-endozoochory		Binomial	77
Seed predation		Binomial	77
Endozoochory		Binomial	77
Exozoochory	Treatment, slug weight, seed weight, movement	Binomial	54
Proportion germinated seeds	Seed source, seed weight, SCP, protein content, oil content	Binomial	147
Proportion germinated feces seeds	Seed weight, SCP, protein content, oil content	Binomial	54
Directional dispersal and endozoochory			
Log(distance shelter-seed)	Treatment	Normal	107

germinated seeds were assessed in GLMMs with seed species as a random effect. Again, the variance in the random effect was close to zero and we therefore used GLMs. A GLM was also run for the proportion of germinated seeds only found in slug feces (endozoochory) with seed weight, seed coat permeability, protein and oil content as fixed effects (Table 1).

Seed dispersal patterns and endozoochory

To identify potential patterns in endozoochorous dispersal, we first performed a Clark–Evans test of aggregation on the point pattern of seeds dispersed in each treatment using the spatstat package (Baddeley and Turner 2005). The aggregation index R provides an indication whether the observed point pattern is clustered or ordered. $R < 1$ indicates a clustered pattern, whereas $R > 1$ assumes an ordered pattern compared to the Null hypothesis of complete spatial randomness. Point patterns of seeds in both treatments were visualized in 2D density plots with contour lines. We further tested the treatment effect on the distance between seeds found in feces and the shelter in a linear model. The distances were log-transformed to achieve a normal distribution.

Results

Slug movement and seed encounter

In 128 of 160 trials, slugs encountered seeds. Video material was sufficient for 65 trials and seeds were encountered in 54 of those trials. The global goodness of fit for the SEM had a Fisher's C of 10.375 with $p = 0.734$ (14 df). Slugs returned to the shelter more often if they spent less time in the shelter before leaving for the first time (Fig. 1b, Supporting information). Slugs in the dry treatment returned to the shelter significantly more than in the wet treatment and also generally when they moved more (Fig. 1b, Supporting information). How much time slugs spent in the shelter was positively affected by the number of times they returned to the shelter (Fig. 1b, Supporting information) and negatively affected by the time slugs spent moving (Fig. 1b, Supporting information). Slugs were more likely to encounter seeds when they moved more, but the chance of seed encounter was not affected by the treatment or the position of the seeds in the microcosm (Fig. 1b, Supporting information).

Slug–seed interactions

In the 128 seed encounters, slugs ingested seeds in 77 trials. Only rarely did slugs ingest all provided seeds, and in the majority of trials, most seeds remained unaffected by slugs (Fig. 2a). Few seeds were dispersed via exozoochory with the highest average of 13.8% for *Trifolium hybridum* and the lowest average of 5.3% for *Medicago sativa*. From all encountered seeds, only 3.5% of *T. incarnatum* seeds were ingested, whereas 48.9% of *M. sativa* seeds were ingested when encountered. Of all ingested seeds, a high proportion was excreted intact and therefore dispersed via endozoochory. The species with the lowest proportion of seeds dispersed via endozoochory was *T. pratense* with 44.8%, whereas 82.5% of *M. lupulina* seeds were dispersed via endozoochory (Fig. 2b). When viewing the fate of ingested seeds on a continuum of negative (seed predation) to positive interactions (endozoochory) for each seed species, seed ingestion resulted mostly in positive interactions for *M.* x *varia* and *M. lupulina* and negative interactions for *T. pratense* seeds. However, seed fate varied considerably within each seed species. Whether any seeds were ingested upon seed encounter was negatively affected by seed weight and seed coat permeability. However, the proportion of ingested seeds was positively affected by the oil content of the seeds (Table 2).

Neither treatment, slug weight nor the tested seed traits affected whether ingested seeds were digested or dispersed via endozoochory (Table 3). A small proportion of seeds was dispersed via exozoochory in the slime trails of slugs (Fig. 2a), but none of the tested variables had a significant effect on exozoochory (Supporting information). There were no differences in the germination success between seeds in slug feces, slime or control seeds. However, seeds with higher seed coat permeability and higher protein content generally germinated better. In the subset of seeds from slug feces, none of the tested seed traits affected germination success (Table 4).

Seed dispersal patterns and endozoochory

We identified locations of seeds dispersed in slug feces (endozoochory) for 48 seeds in the dry treatment and 59 seeds in the wet treatment. Point patterns of seeds in both treatments showed a clustered pattern (Fig. 3), with seeds clustered around the location of the shelter. However, 2D density plots indicate that the clustering of seeds around the shelter in the dry treatment was denser than in the wet treatment and a linear model showed a strong effect of treatment on the distances between seeds and the shelter. Seeds in the wet treatment were significantly further away from the shelter than seeds in the dry treatment (estimates [wet treatment] = $0.73 \pm 0.203^{***}$, R^2 adjusted = 0.101). We recorded dispersal distances and gut retention times in 12 and 18 trials, respectively, with the maximum dispersal distance of 32.5 m and a mean of 14.4 m . Gut retention time averaged 13 h but varied between 1.5 and 31 h.

Discussion

Slug movement and seed encounter

Slug activity is strongly driven by ambient temperature and soil moisture (Willis et al. 2006) and slugs reduce their activity and movement to conserve energy and reduce water loss in adverse conditions (Prior 1985, Nicolai and Ansart 2017). Contrary to our expectations, unfavorable dry conditions did not decrease the proportion of time slugs spent moving nor were slugs in the dry treatment more reluctant to leave their shelter. In our experiment, ambient temperature, humidity

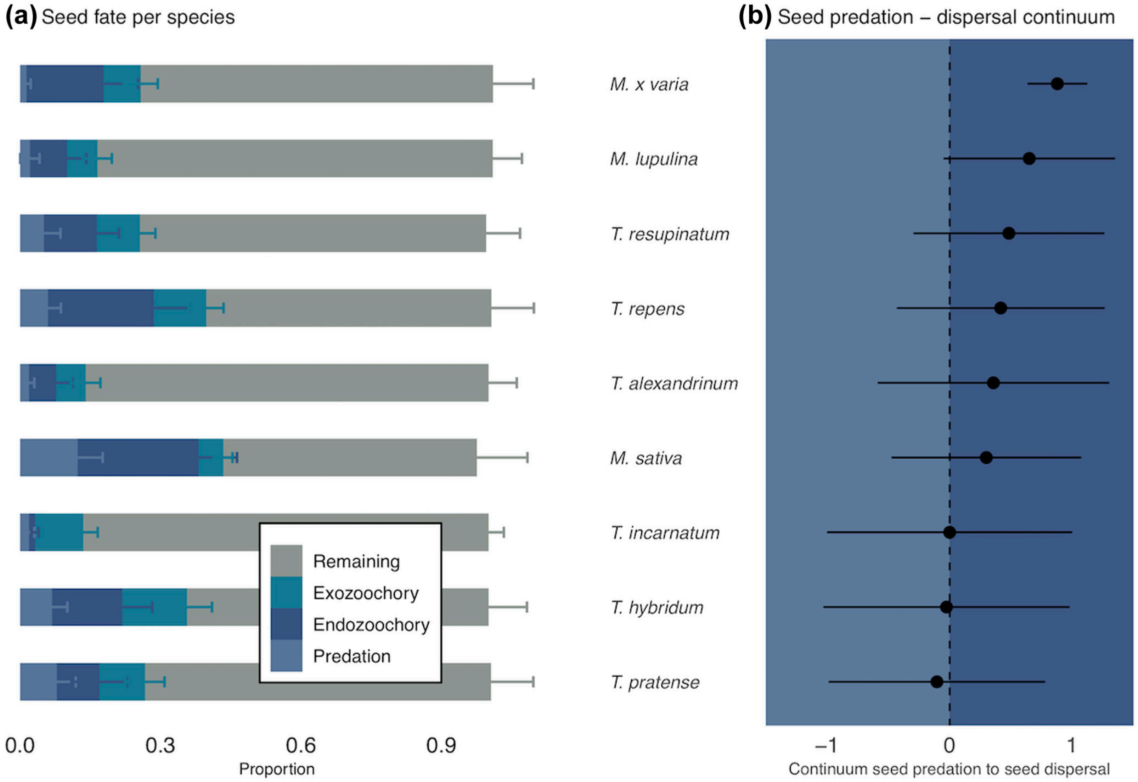

Figure 2. Overall seed fate of legume seeds encountered by slugs (a) and seed fate of ingested seeds (b). (a) Stacked bar plots with standard errors for the proportions of seed fate in slug–seed interactions for each plant species. (b) Ingested seeds of each species on a continuum of negative (seed predation) to positive interactions (endozoochory). The mean and standard errors are calculated as the proportion of seeds that were excreted intact (endozoochory) minus the proportion of seeds that were digested (seed predation).

and shelter moisture were the same for both treatments, only the surface moisture varied between treatments. It is therefore likely that slugs in the dry treatment did not suffer from moisture loss if they returned to their shelter more frequently, which we confirmed in the structural equation model. This also supports Rollo and Wellington (1981), who postulate that homing behavior is especially important in unfavorable environmental conditions.

Interestingly, the initial time until leaving the shelter was also not affected by surface conditions or the slug's weight and we therefore assume that the slug's individual behavior is more important for this action. Boldness has been shown to be a decisive trait in the dispersal of the snail *Cornu aspersum* (Dahirel et al. 2017) and it might therefore be possible that individual slugs also exhibit different levels of boldness that determine their exploratory movement. There is also evidence that *Arion lusitanicus* shows different behavior responding to a variety of stimuli compared to native slugs (Kappes et al. 2012). In our study, we did not test individual-based effects

Table 2. Effects of treatment, slug weight and seed traits on seed ingestion. Given are odds ratios and confidence intervals (CI) based on binomial GLMs. Significant effects ($p < 0.05$) in bold.

	Ingestion (yes/no)		Ingestion (proportion)	
Predictor	Odds ratio	CI	Odds ratio	CI
(Intercept)	1.21	0.69–2.14	**0.22**	**0.11–0.44**
Treatment [wet]	1.85	0.82–4.16	1.26	0.51–3.09
Slug weight	1.41	0.89–2.24	1.15	0.76–1.72
Seed weight	**0.53**	**0.34–0.84**	0.59	0.32–1.09
SCP	**0.55**	**0.33–0.92**	1.18	0.71–1.98
Protein content	0.85	0.53–1.36	1.01	0.47–2.18
Oil content	1.03	0.64–1.65	**1.79**	**1.01–3.18**
Observations	128		128	
R^2 Tjur	0.178		0.048	

Table 3. Effects of treatment, slug weight and seed traits on the proportion of ingested seeds that was digested (seed predation) or excreted intact (endozoochory). Given are odds ratios and confidence intervals (CI) based on binomial GLMs. Significant effects ($p < 0.05$) in bold.

	Seed predation		Endozoochory	
Predictor	Odds ratio	CI	Odds ratio	CI
(Intercept)	0.36	0.16–0.80	**2.76**	**1.25–6.07**
Treatment [wet]	2.06	0.75–5.63	0.49	0.18–1.33
Slug weight	0.97	0.59–1.61	1.03	0.62–1.71
Seed weight	0.99	0.56–1.77	1.01	0.57–1.78
SCP	0.77	0.42–1.41	1.29	0.71–2.36
Protein content	1.14	0.63–2.07	0.88	0.48–1.59
Oil content	0.74	0.41–1.34	1.34	0.75–2.41
Observations	77		77	
R^2 Tjur	0.015		0.015	

Table 4. Effects of seed source (control, slug slime and slug feces) and seed traits on the proportion of germinating seeds overall and only for seeds found in feces. Given are odds ratios and confidence intervals (CI) based on binomial GLMs. Significant effects ($p < 0.05$) in bold.

	Germination		Germination feces seeds	
Predictor	Odds ratio	CI	Odds ratio	CI
(Intercept)	**0.00**	**0.00–0.04**	0.00	0.00–2758.36
Source [feces]	0.47	0.21–1.05	–	–
Source [slime]	1.26	0.42–3.75	–	–
Seed weight	0.68	0.41–1.12	0.76	0.32–1.84
SCP	**1.03**	**1.00–1.05**	1.02	0.98–1.05
Protein content	**1.39**	**1.07–1.79**	1.29	0.79–2.13
Oil content	1.00	0.83–1.19	0.97	0.73–1.29
Observations	147		54	
R^2 Tjur	0.015		0.000	

and did also not identify slugs to species level. It could well be that we used different *Arion* species in our experiment, as they are hard to distinguish based on morphological traits and they also hybridize (Rowson et al. 2014, Zemanova et al. 2017). Responses due to individual slug traits and/or actual species differences might have therefore disguised actual treatment effects on movement.

It is not surprising that the chance of seed encounter increased when slugs moved more. Slugs move in a random, meandering pattern when exploring their surroundings (Rollo and Wellington 1981) and we also observed this in the videos of the trials. With more exploratory movement, chances of seed encounter naturally increase, independent of environmental conditions.

Slug–seed interactions

In our experiments, slugs ingested seeds of all species but preferred seeds that were smaller and had a lower seed coat permeability. Numerous studies confirm a relationship between seed ingestion and seeds size and/or weight for slugs and other invertebrates (Türke et al. 2010, Calvino-Cancela and Rubido-Bará 2012, Türke and Weisser 2013, Kulkarni et al. 2015, Clause et al. 2017). However, compared to carabids, slugs do not destroy the seeds but rather only scrape them with their radula (Jennings and Barkham 1976) and then swallow them whole, which can explain their preference for smaller seeds. We can only speculate on the role of seed coat permeability for initial seed ingestions. In an experimental study, hamsters were able to detect hidden seeds that emitted olfactory cues much better than seeds without cues (Paulsen et al. 2013). The authors argue that hard-seededness (physical dormancy), which corresponds to low seed coat permeability, prevents seeds from releasing volatile compounds to avoid detection by mammalian predators, but this hypothesis has recently been disputed (Jaganathan 2018). Slugs can detect food sources using olfactory cues (Kiss 2017), so according to Paulsen et al. (2013) we could expect that they would detect seeds with higher seed coat permeability but the opposite was the case. We are therefore not able to tell whether emitted volatile compounds play a role in seed detection in our study. However, seed coat permeability is also closely linked to seed coat structure (Ma et al. 2004) and perhaps specific seed coat structures in species with lower seed coat permeability were more attractive to slugs.

Once slugs started to consume seeds, they consumed larger amounts of high quality seeds, i.e. seeds with higher oil content. This has also been shown for the common earthworm *Lumbricus terrestris*, where seed ingestion was largely driven by the oil content of seeds (Clause et al. 2011, 2017). Slugs assess the quality of and the preference for food by odor and taste (Sahley et al. 1981). We assume that they are able to identify preferred oil-rich seeds by taste since oil content did not affect initial seed ingestion.

We need to emphasize that we worked with a no-choice experiment, which might distort the results and hence our interpretation. Peters et al. (2000) showed that slugs consumed different amounts of leaves and also showed different preferences in choice versus no-choice experiments. Whereas in the no-choice experiments, slugs seemed to prefer legume leaves, this preference was not apparent in choice experiments, where slugs varied their diet considerably. However, the preference for oil-rich seeds we observed might persist even in choice experiments. Earthworms preferred oil-rich seeds when being given the choice of different seed species (Clause et al. 2011), and it is plausible that slugs behave similarly.

None of the investigated seed traits affected the fate of ingested seeds but we have to acknowledge that we used seeds from one plant family with rather small variation in the selected traits. For example, oil content ranged between 4.3 (*T. incarnatum*) and 11.9% (*M.* x *varia*), whereas oil content of seeds in the study by Clause et al. (2017) ranged between 0.9 (*Lolium perenne*) and 34.9% (*Urtica dioica*). In our study, protein content varied between 34.2 (*T. alexandrinum*) and 40.95% (*T. incarnatum*) compared to the much larger range used by Clause et al. (2017) (14.3 (*L. perenne*)–41.8% (*Ononis spinosa*)). Even with the larger range of oil and protein content, Clause et al. (2017) did not detect significant effects of those traits on the digestion of seeds ingested by different earthworm species.

The majority of consumed seeds were excreted intact, leading to a net-positive outcome for seeds of most species (Fig. 3b). As stated earlier, slugs ingest seeds whole and once seeds reach the digestive tract, they are not exposed to any mechanical destruction (Calvino-Cancela and Rubido-Bará

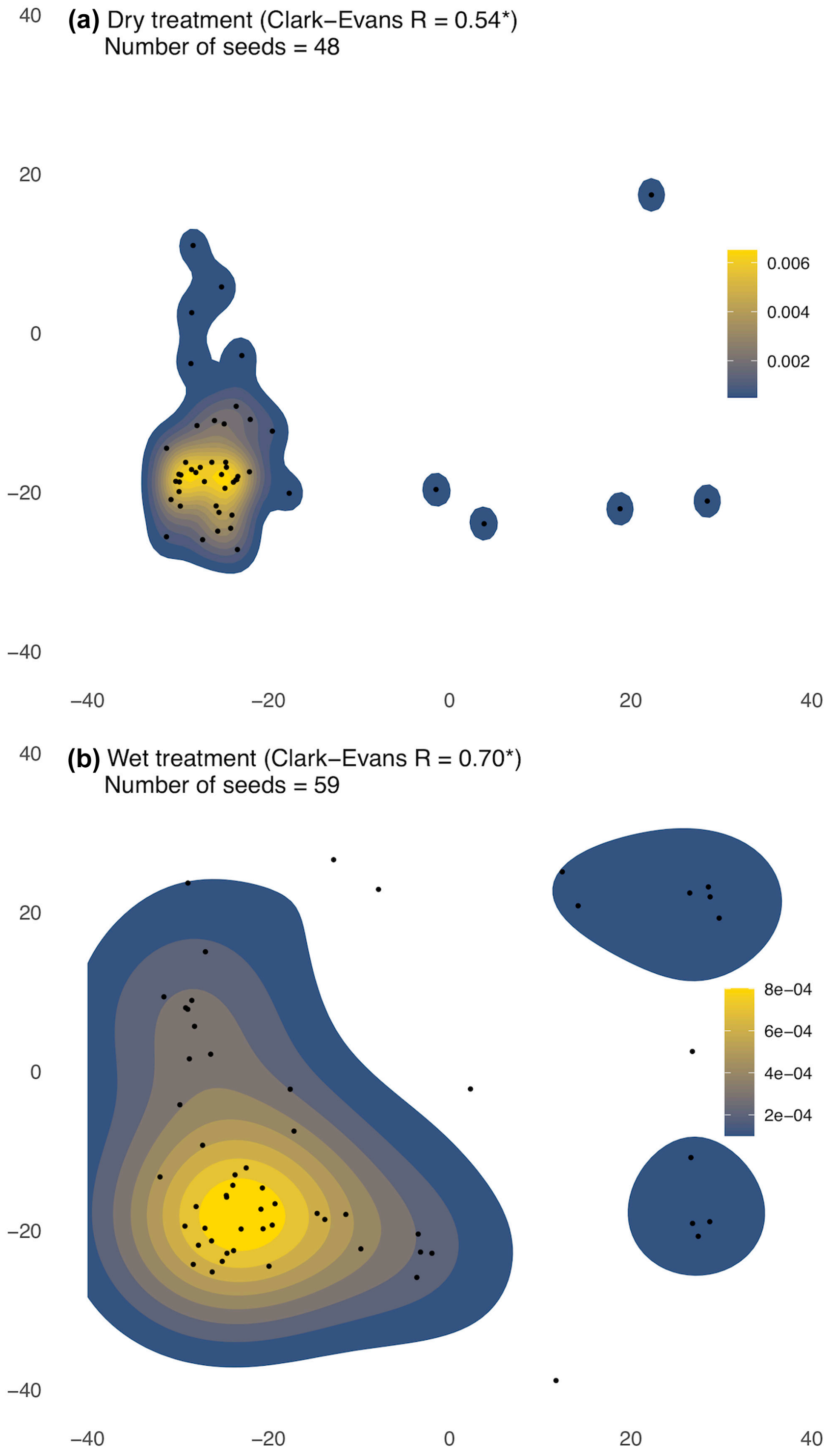

Figure 3. 2D density plot of seeds dispersed via endozoochory in the dry (a) and wet (b) treatment. The Clark–Evans R < 1 (*p-value < 0.05) indicates a clustered pattern in locations of seeds in both treatments. The kernel density estimation is given in differently colored contour bands.

2012). Calvino-Cancela and Rubido-Bará (2012) report similar results of high proportions of endozoochory for different plant species. The evidence is mounting that we should therefore see slugs as successful endozoochorous seed dispersers rather than only seed predators – at least for smaller-seeded species. In contrast to Calvino-Cancela and Rubido-Bará (2012), we could not observe a positive effect of gut passage on seed germination. However, germination rates across our tested species were relatively high and seeds germinated very quickly (often within one or two days). We even found some seeds that had already germinated in the feces during the trial. Not surprisingly, seed coat permeability affected germination of seeds positively, irrespective of whether these seeds were excreted after gut passage, found in slime trails or germinated under controlled conditions. Seed coat permeability affects imbibition, which is an important stage in seed germination (Woodstock 1988). Seed species with higher protein content also had higher germination rates, confirming the important role of storage proteins for germination (Ashton 1976).

Seed dispersal patterns and endozoochory

Directed dispersal of seeds does not only entail the transport of seeds by specific dispersal agents to non-random locations but these locations should also provide better conditions and hence lead to higher recruitment than in other sites (Howe and Smallwood 1982), either through escape from predators and/or better germination conditions. Our results are the first showing that endozoochory by slugs fulfills the first requirement for directed dispersal – transport of the seeds to a non-random location. Additionally, we observed that different environmental conditions do not only affect slug activity and movement behavior, but also have subsequent effects on dispersal patterns of ingested seeds.

Some studies estimate potential dispersal distances by relating gut retention time to moved distances (Türke et al. 2010, Calvino-Cancela and Rubido-Bará 2012). When combining those potential maximum distances with meandering slug movement patterns (Rollo and Wellington 1981), estimated home ranges and location fidelity (Grimm and Paill 2001, Forbes et al. 2020, Nyqvist et al. 2020), dispersal distances most likely do not exceed a few meters or up to tens of meters, which our observations also confirm. Calvino-Cancela and Rubido-Bará (2012) hence argued that slugs are not very effective dispersers – at least for seeds that are usually dispersed by vertebrates. However, we argue that even the relatively short distances already reduce competition with siblings and dispersal by slugs also has the advantage of directed dispersal to a favorable microhabitat (moist shelter). Directed dispersal by invertebrates is mostly know for ants (Hanzawa et al. 1988, Leal et al. 2007) or dung beetles (Andresen and Levey 2004, Nichols et al. 2008) but as far as we are aware, this is the first reported observation of potential directed dispersal via endozoochory by an invertebrate. Directed dispersal can be advantageous by increasing germination and/or survival probability of seedlings (Hanzawa et al. 1988, Andresen and Levey 2004, Leal et al. 2007). Calvino-Cancela and Rubido-Bará (2012) showed that slug gut passage increases germination for some plant species and Türke et al. (2010) observed that *Anemone* sp. seeds were less attractive to rodent seed predators after slug gut passage. For some seed species, it might therefore be highly advantageous to be ingested by slugs, since this could not only improve their germination success but also increase seedling survival by depositing seeds in moist and dark microhabitats and deter seed predators. Especially under adverse environmental conditions, i.e. drought periods that are predicted to increase in Europe (Spinoni et al. 2018), this might be decisive for successful germination of some plant species. We observed that homing behavior in slugs increased under dry surface conditions and the clustered pattern of dispersed seeds around the shelter was much more pronounced. This would suggest that in dry conditions, ingested seeds are even more likely to be dispersed to potentially favorable sites.

Slugs spend considerable time in their shelter and often return to shelters (Edelstam and Palmer 1950, Gelperin 1974, Rollo and Wellington 1981, Ng et al. 2013, this study). They also prefer seedlings as valuable resources (Honek et al. 2009, Strauss et al. 2009). This most likely increases the risks of newly emerging seedlings to be consumed by slugs in and around shelters, negating positive effects of directed dispersal. Although the range of seed traits in this experiment was low, we detected a preference for small and oil-rich seeds. Depending on the plant community and seed availability at given times, preferential ingestion and subsequent endozoochory might lead to a spatial re-structuring of the community through directed dispersal of specific species, which can be enforced by dry environmental conditions. However, spatial re-structuring can only occur when the directed dispersal to favorable microhabitats poses an advantage for the seed and does not increase predation risks for seedlings, which will lead to a net negative outcome for the plant species. Even though endozoochorous dispersal by slugs corresponds to the first requirement of directed dispersal (dispersal to specific location), it remains to be tested whether this truly is directed dispersal by providing advantageous conditions for plant recruitment.

Acknowledgements – We thank S. Schulte and M. Fabi for their help in analysing video material.
Funding – This work was supported by the Research Innovation Fund of the University of Freiburg (Project number: 2100132201).
Conflicts of interest – The authors have no conflicts of interest.

Author contributions

Gesine Pufal: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (supporting); Methodology (equal); Project administration (lead); Writing – original draft (lead); Writing – review and editing (equal). **Carl Skarbek**: Investigation (lead); Methodology (equal); Writing – review and editing (equal).

Data availability statement

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.qrfj6q5d5> (Pufal and Skarbek 2021).

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