Complicating a Complex Ecosystem Function: the Controversial Role of Gastropods in a Myrmecochorous Seed Dispersal Mutualism

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“After decades of studies on seed dispersal by animals, we are still ignorant about which seed-dispersing species are obligatory dispersers for the survival of many plants.”

From “Why seed dispersers matter”, an interview with Pierre-Michel Forget (mongabay.com; March 07, 2010)

Red slug (*Arion rufus*) swallowing a seed of European wild ginger (*Asarum europaeum*). (Photographs by M. Türke, Jena, 2010)

CHAPTER ONE

General Introduction

The dispersal of diaspores of plants is a function provided by the facilities of an ecosystem and may impact on plant distribution and migration and consequently also on plant species richness and abundance. I focused in my work on the seed fate of supposedly ant-dispersed plants (myrmecochores) in Central European beech forests, where these herbs are quite numerous concerning individuals and species as well. However, previous studies indicated that ants are less abundant in mature shaded beech forest stands due to low temperature and high moisture and thus seeds might be limited in dispersal and may fall prey to seed predators instead. In a series of field seed removal and laboratory feeding experiments, it turned out quickly that gastropods may play a crucial role in seed removal and dispersal of these herbs. Therefore, I started to investigate this previously undescribed seed dispersal mutualism in more detail. In the following I will present some general aspects of seed dispersal, followed by a summary on seed dispersal activities of ants (myrmecochory) and of gastropods (gastropodochory) and, finally, I will discuss seed dispersal of myrmecochores in the context of the abundance of the dispersers (ants and gastropods) in European beech forests.

Seed Dispersal – an Ecosystem Function

Most vascular plant species produce sexual diaspores for reproduction and dispersal. Seed dispersal is an essential force driving plant migrations, ecological successions of vegetations and, considering an unequal potential for establishment and dominance of different plant species, varying dispersal abilities can promote plant species diversity in a heterogeneous, gap-rich landscape (Brunet and von Oheimb 1998b; Dzwonko and Loster 1992; Ehrlen and Eriksson 2000; Fenner and Thomson 2005; Müller(-Schneider) 1955). A body of growing interest and research in ecology deals with the problems that arise from plant invasions and the reaction of native plants to climate change and these issues are closely related to the

dispersal ecology of plants (Buckley et al. 2006; Higgins and Richardson 1999; Wilson et al. 2009).

From a seed's or seedling's point of view most sites of the landscape are unsuitable for establishment and the environment is hostile, especially in the vicinity of the parent plant (Vander Wall et al. 2005). Therefore, plants have evolved sundry dispersal modes to transport seeds actively or passively to suitable microsites. Such dispersal vectors include wind, water, ballistic powers, gravity, man or animals such as birds, mammals, reptiles and invertebrates with ants as the most important group, and many plants make use of several dispersal modes within the same seed or by the production of different seed types (polychory, mostly diplochory) (Forget et al. 2005; Müller(-Schneider) 1955). The facilities of an ecosystem define the predominant dispersal modes, e.g. the availability of animals, water or wind for dispersal (Müller(-Schneider) 1955; Sernander 1906; Ulbrich 1919). Animals can be effective dispersers, but their presence and abundance is often closely related to the conditions of an ecosystem. Anthropogenic-driven alterations of ecosystems can lead to the loss of these dispersers, which may have detrimental effects on the plant species lacking dispersal (Christian 2001; Cordeiro and Howe 2003; Herrera and Garcia 2010; Traveset and Richardson 2006).

Prior to or after initial dispersal, seeds may face a variety of possible destinies, including death due to various hazards (predation, loss to pathogens, fatal germination at depth, loss of viability with age), secondary dispersal, dormancy or germination (Fenner and Thomson 2005; Vander Wall et al. 2005). Again, the ecosystem has an impact on the fate of seeds. Seed dispersal can be limited by the availability of dispersers or by the high abundance of seed predators (Fenner and Thomson 2005; Forget et al. 2005; Garcia et al. 2005).

Myrmecochory – Seed Dispersal by Ants

Most plants dispersed by animals produce fleshy fruits which are consumed together with the seeds therein and seeds will have to pass the guts of the animals undamaged to gain dispersal success. In ant-dispersed plants, called myrmecochores, the seeds are normally not swallowed but are transported by ants with the mandibles to their nest, where a nutrient-rich appendage, the elaiosome, is consumed, while the seed itself is discarded (Beattie 1985; Gorb and Gorb 2003; Sernander 1906). This behavior could be considered as deliberate removal of seeds (Cousens et al. 2008) and was also called stomatozoochory (Müller(-Schneider) 1955).

Research on the seed dispersal by ants (myrmecochory) dates back to the 19th century (see references in Sernander (1906)) and was first intensively studied by Sernander

(1906) with important insights into the evolutionary ecology of this mutualism reviewed by Beattie (1985). Meanwhile, several hundred studies have been published on this topic, mainly focusing on the benefits of myrmecochory for the plants and only rarely on benefits for the ants. The number of plant species that are considered as being ant-dispersed is increasing constantly and recent estimates assume that more than 11,000 species worldwide are myrmecochores (Lengyel et al. 2010). Myrmecochory is spread almost all over the globe and in certain habitats it may account for a great proportion of the herbaceous biomass and species, highlighting the ecological importance of this dispersal mode (Beattie 1985; Lengyel et al. 2010).

Myrmecochores show a series of adaptations to ants, called the myrmecochorous syndrome (Gorb and Gorb 2003; Ulbrich 1919). Probably most important is the presence of the elaiosome on diaspores, which contains fatty acids, amino acids, carbohydrates and vitamins (Fischer et al. 2008) and is consumed by the ants or fed to the larvae (Fischer et al. 2005). Other traits which may be exhibited by a myrmecochore include early-spring-flowering to synchronize fruit production with the nutritional needs of ants at that time (Guitian and Garrido 2006) or the bending of stalks to the ground to bring ripe diaspores into the reach of ants (Ulbrich 1919).

Five hypotheses have been proposed and tested experimentally on where the selective advantage of myrmecochory for plants may be seen and each hypothesis could be supported at least in some studies (Beattie 1985; Giladi 2006). However, often only one or a few hypotheses are tested, but several might apply to certain plant species (Giladi 2006). These hypotheses include the assumptions that dispersal transfers the seed to a nutrient rich environment (within the ant nest) or that it helps seeds to avoid fire, predation or competition between relatives or species.

Diaspores are unequally attractive to ants, depending on size, the elaiosome-diaspore ratio or chemical content (Boulay et al. 2006; Gorb and Gorb 2003). Different ant species may exhibit different preferences for certain diaspores and this preference can depend on foraging strategies or diet or can be related to morphological restrictions, especially the size of ants or the span of the mandibles (Beattie 1985; Boulay et al. 2007b; Gorb and Gorb 2003; Ness et al. 2004; Ness et al. 2009; Peters et al. 2003; Servigne and Detrain 2008). Ants may also differ in the benefit they provide for the plants, in particular, the effectiveness of seed dispersal (Gomez and Espadaler 1998a; Ness et al. 2004; Pudlo et al. 1980). Several studies report on differential dispersal and seed handling behavior of co-existing ant species and demonstrate that a diverse ant fauna can have positive effects on the dispersal pattern of myrmecochores (Gomez and Espadaler 1998b; Gomez et al. 2005; Gorb and Gorb 2003; Pudlo et al. 1980). Other studies, in contrast, propose that most seed

removal was due to only a few ant species of the whole ant community (Boulay et al. 2007b; Gove et al. 2007; Manzaneda and Rey 2009; Ness et al. 2009).

While plants appreciate if their diaspores are collected by most ant species which consume elaiosomes after dispersal, other animals are attracted as well but are not welcome to do so, as most of them destroy the seed or consume the elaiosome without dispersal of the seed. Rodents and insects, mainly ground beetles, have been shown to feed on diaspores or elaiosomes (Heithaus 1981; Higashi and Ito 1991; Kjellsson 1985; Ohkawara et al. 1996). Also gastropods have been observed feeding on elaiosomes (Gunther and Lanza 1989; Mesler and Lu 1983) and it has been demonstrated several times that they act as seed dispersers of non-myrmecochorous plants (see next chapter), emphasizing their potential for dispersal of myrmecochores.

Gastropodochory – Seed Dispersal by Slugs and Snails

Plant species are often differently palatable to gastropod herbivores and plants may profit from the presence of slugs or snails if their competitors suffer more from herbivory than they do (Buschmann et al. 2005; Frank 2003; Lanta 2007). That gastropods may benefit plants directly by the dispersal of their diaspores, is much less well known and documented. The fact that diaspores are dispersed by gastropods is not new but literature on this topic has remained scarce. Research on the predation of mostly soft or imbibed seeds by gastropods has been only slightly more comprehensive (e.g. Barker 2001; Cardina et al. 1996; Gebauer 2002; Simonetti 2003).

Müller(-Schneider) (1934) was the first to test frugivory by gastropods in field and laboratory experiments and he proposed the term gastropodochory for seed dispersal by terrestrial gastropods and gastropodochores for the plants involved (Müller-Schneider 1967; Müller-Schneider and Lhotská 1971). His studies were motivated by a comment of M. W. Beyerinck on the dispersal of strawberries (*Fragaria*) by gastropods published some years before (Ridley 1930). Müller(-Schneider) (1934) repeatedly observed gastropod feeding traces on fruits of herb Paris (*Paris quadrifolia* L.), on raspberries and blueberries in forests. His assumption that slugs and snails feed on a variety of fleshy fruits and swallow the small seeds therein was confirmed by laboratory feeding trials. The seeds of 13 fleshy fruited herbs or shrubs passed the guts of red slugs (*Arion empiricorum* Férussac synonym *Arion rufus* L.), Burgundy snails (*Helix pomatia* L.) and *H. arbustorum* L. (synonym *Arianta arbustorum* L.) undamaged within ten to twelve hours. Seed germination of control seeds did not differ from seeds that had passed gastropod guts. Interestingly, germination in bilberry (*Vaccinium myrtillus* L.) was even accelerated after gut passage. The potential role of banana slugs (*Ariolimax columbianus* Gould) as seed dispersers of different fleshy fruited Pacific

Northwest plant species has also been confirmed (Gervais et al. 1998). The influence of gut passage on germination was species-specific. Germination of Salmonberry (*Rubus spectabilsi* Pursh) was reduced after gut passage and, interestingly, seeds of differently colored fruit morphs differed in their germination rates.

However, these findings on frugivory and seed dispersal by gastropods have received rather little attention. Plant species with fleshy fruits are usually adapted to dispersal by vertebrates, which indeed will provide much greater dispersal distances than gastropods could do. Solely the plant moschatel (*Adoxa moschatellina* L.) could be an exception. During the ripening process of the small yellowish-greenish fruits, the stalk bearing the fruits slowly bends to the ground and might even hide its fruits beneath the leaf litter (Müller-Schneider 1967), similar to ant-dispersed plants (Ulbrich 1919). However, seeds remain within the fruits, counteracting seed dispersal by ants. Furthermore, different seed-collecting ant species rejected the seeds of moschatel (Müller-Schneider 1967). And it is unlikely that birds feed on the fruits due to color and presentation of the fruits and it was not observed by Müller-Schneider (1967). In contrast, slugs and snails readily consumed fruits in the field and under experimental conditions and defecated germinable seeds. Müller-Schneider (1967) suggested that moschatel like no other plant in Central Europe is adapted to seed dispersal by gastropods. The role of small mammals for the dispersal of moschatel, however, has not yet been assessed.

In addition to frugivory and subsequent seed dispersal, gastropods have also been shown to disperse lichens endozoochorously (Boch et al. 2011; Fröberg et al. 2001; McCarthy and Healy 1978). However, seed dispersal of plants without fleshy fruits by gastropods is sparse and findings are often based on anecdotal evidence as almost no studies have been conducted to test this phenomenon. Frömmig (1950) found a single undamaged seed of caraway (*Carum carvi* L.) in the feces of a wild-caught red slug. Kollmann and Bassin (2001) reported that slugs removed a fair proportion of gold-of-pleasure (*Camelina sativa* (L.) Crantz) seeds in wildflower strips in Switzerland by feeding and even observed germination of seeds from slug feces. For the anemochore dandelion (*Taraxacum* spp.), a contribution of gastropods to seed removal was found in field seed removal experiments (Honek et al. 2009). Ten out of twelve slug and snail species consumed seeds in the laboratory and seeds passed the guts of gastropods undamaged. The germination rate of seeds defecated by the Spanish slug (*Arion lusitanicus* Mabille) was high, though by 20% reduced in comparison to controls (Honek et al. 2009). However, the authors found no seeds in feces of wild-caught slugs.

To my knowledge, there are only two studies describing gastropod feeding on seeds of ant-dispersed (myrmecochorous) plants previous to my own observations. In maple-beech woods in New York, USA, Gunther and Lanza (1989) observed slugs feeding on seeds of

myrmecochorous *Trillium* spp. Unfortunately, they did not describe the feeding itself nor specified the slugs involved. Mesler and Lu (1983) also documented active foraging of banana slugs on seeds of *Trillium ovatum* Pursh. Slugs knocked down seeds from plants and foraged for elaiosomes. Seeds were transported over short distances by slugs, adhering to their mucus (Mesler and Lu 1983). Such epizoochorous dispersal by gastropods was also described for lichen fragments (Bailey 1976) and asexual brood branches of the moss *Dicranum flagellare* Hedw. (Kimmerer and Young 1995). But endozoochorous dispersal of myrmecochorous seeds has not yet been described.

Myrmecochores and Ants: a Beech-Forest-Paradox?

As demonstrated before, seed dispersal impacts on many ecological relevant aspects of plant communities and landscapes such as plant distributions and diversity. Migration rates of many forest herbs are low, often reaching only one metre per year or even less, and thus, these species, including many myrmecochores, are often absent in recent forests and are used as indicator plants for ancient woodlands (Brunet and von Oheimb 1998a; Brunet and von Oheimb 1998b; Dzwonko and Loster 1992; Ehrlen and Eriksson 2000; Godefroid et al. 2005; Orczewska 2009; Orczewska 2010; Verheyen and Hermy 2001; Wulf 1997).

Most deciduous or coniferous mesic forests harbor a diverse community of myrmecochores, as is also the case in Central European beech forests (Beattie 1985; Beattie and Culver 1981; Handel et al. 1981; Sernander 1906; Ulbrich 1919). In contrast to most other forest types, however, mature shaded forests dominated by European beech (*Fagus sylvatica* L.) are unsuitable habitats for most ant species and the few ants living in these forests, mainly the red ant (*Myrmica ruginodis* Nyl) and the arboreal brown tree ant (*Lasius brunneus* Latreille), persist at extremely low nest densities (Schlick-Steiner and Steiner 1999; Seifert 1986; Seifert 2007; Wlodarczyk 2010). This is due to low temperature, high moisture and the lack of nesting sites (smooth bark of beech) in beech forests (Seifert 1986). In a dry oak forest in East Germany, for instance, ant nest density was estimated as 223.6 nests/ 100m^2, whereas in two different beech forests in East Germany, nest densities were estimated as 0.2 and 0.5 nests/ 100m^2, respectively (Seifert 1986). Thus, myrmecochore diversity and cover and the abundance of their seed dispersers, ants, appear to be negatively correlated in mature beech forests.

A similar pattern was found in one of the first and fundamental studies correlating the abundance and species richness of myrmecochores with the abundance of ants in 10 different forest types of West Virginia, USA (Beattie and Culver 1981). Ant abundance was generally positively correlated with the number of myrmecochorous species in these forests. There were, however, exceptions to this general pattern: while the number of

myrmecochores in a forest dominated by American beech (*Fagus grandifolia* Ehrh.) was similar to the other nine deciduous and coniferous mixed forest types, very few ants (only one observation of ants on tuna baits) were observed in this forest. In another forest type dominated by red spruce (*Picea rubens* Sarg.) and yellow birch (*Betula alleghaniensis* Britt.) a single myrmecochore, the dimpled trout-lily (*Erythronium umbilicatum* Parks & Hardy) accounted for the highest myrmecochore density of all forests and made up 75% of all herb stems in this forest, but not a single ant was observed. Unfortunately, the authors did not discuss the ecological implications of their findings for the myrmecochore communities in the forests lacking ants. In another study, however, seed dispersal was found to be ant-limited in dense populations of myrmecochores (Smith et al. 1989). If not dispersed by ants, seeds or elaiosomes may be consumed by predators (Heithaus 1981; Ohara and Higashi 1987; Ohkawara et al. 1996).

Thus, one could suggest that an overwhelming majority of myrmecochorous seeds in Central European beech forests might fall prey to seed predators and plants will migrate by vegetative spread rather than by seed dispersal if ants are rare or absent. However, migration rates of forest myrmecochores regularly exceeded the very low annual vegetative spread, indicating seed dispersal over short distances (Brunet and von Oheimb 1998a; Brunet and von Oheimb 1998b; Dzwonko and Loster 1992; Orczewska 2009; Orczewska 2010; Verheyen and Hermy 2001). Thus, I have posed the question, if animals other than ants could provide seed dispersal of myrmecochores in beech forests, too?

Goal of the study

In my studies, I have assessed the fate of myrmecochorous seeds in mature shaded beech forests. Field work was conducted in experimental plots within the framework of the Biodiversity Exploratories project (www.biodiversity-exploratories.de, Fischer et al. (2010)), investigating the relationship between land use, biodiversity and ecosystem functioning in a large-scale and long-term approach. The experimental plots comprise 150 100 × 100 m forest plots of varying management types, including 104 stands dominated by European beech, which are distributed over three regions: (1) the UNESCO Biosphere Reserve Schorfheide-Chorin in North-eastern Germany (13°76’ E/ 53°00’ N), (2) the National Park Hainich and its surrounding areas (Hainich-Dün) in Central Germany (10°47’ E/ 51°15’ N), and (3) the UNESCO Biosphere Area Schwäbische Alb (Swabian Jura) in South-western Germany (9°39’ E/ 48°43’ N). Seed removal experiments in mature beech forests indicated a significant contribution of gastropods to the seed removal of myrmecochorous forest herbs (chapters 2 & 5). Thus, I investigated the seed dispersal or predation potential of gastropods in seed feeding experiments (chapters 2, 3 & 4). In addition, I collected more than 300 red

slugs (*A. rufus*) in beech forests and searched for seeds in their feces (chapter 2). As it turned out, gastropods appear to act as seed dispersers, and therefore I was further interested in the benefits they might provide for the plants. A germination experiment with seeds of wood anemone (*Anemone nemorosa* L.) was conducted to see whether gastropod-dispersed seeds are still viable (chapter 2). I also assessed retention times of seeds in the guts of *A. rufus* individuals and based on the results of this experiment, I conducted a dispersal experiment with marked slugs in the forest to get an idea of how far they might transport seeds (chapter 2). I also tested whether seeds that had been swallowed and defecated or of which elaiosomes had been consumed by slugs were less attractive to rodents in the laboratory (chapter 2) and whether they were less likely to be removed by animals from seed depots in forests (chapter 3). To understand the underlying mechanisms that influence the seed feeding behavior of gastropods, I tested for an influence of seed size (chapter 3) and of gastropod size on the seed dispersal potential (chapters 2, 3 & 4). To allow speculations on the generality of the finding that gastropods consume and disperse myrmecochorous seeds, with a special focus on beech forests, I expanded the field seed removal experiments from a local to a large geographical scale (chapter 5) and offered seeds of a number of plant species to several gastropod species in laboratory feeding trials (chapter 3). With the help of pitfall trap samples of ants and vegetation surveys, I tested for the assumption that beech forests that contain many myrmecochores harbor only few ants (chapter 5). To summarize, the following questions should be answered with the abovementioned experiments and surveys:

(1) Are gastropods acting as seed predators or dispersers? (chapters 2 & 3)
(2) Which gastropod species are rather elaiosome predators, which are seed dispersers? (chapters 2, 3 & 4)
(3) Are there differences among plant species? (chapters 2 & 3)
(4) Which benefits might plants gain from gastropodochory? (chapters 2 & 3)
(5) Could the body size/ mass of slugs influence their seed dispersal potential? (chapters 2,3 & 4)
(6) What is the relationship of myrmecochores and ants or gastropods in beech forests? (chapters 2 & 5)
(7) What is the fate of myrmecochorous seeds in beech forests and could gastropods substitute ants as dispersers? (chapters 2 & 5)

Manuscript Overview and Author Contribution

CHAPTER TWO

Seed Consumption and Dispersal of Ant-Dispersed Plants by Slugs

Manfred Türke • Eric Heinze • Kerstin Andreas • Sarah M. Svendsen • Martin M. Gossner • Wolfgang W. Weisser

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After I made observations of slugs feeding on seeds in the field and in the laboratory, I designed a series of experiments to investigate the role of gastropods for seed dispersal. Here, we conducted a field seed removal experiment in the Hainich National Park, seed feeding experiments with gastropods in the laboratory, seed feeding experiments with slug-consumed seeds with rodents, a slug dispersal experiment and a germination experiment and we assessed gut-passage times for seeds in slugs.
I designed all experiments, collected seeds and gastropods, analyzed the data, wrote the first draft of the manuscript and took part in and supervised all experiments. I also conducted a series of field seed removal experiments which preceded the one published here, and which are not published but helped to improve the methods used, the seed feeding experiments with gastropods, the assessment of gut-passage times and some of the experiments mentioned in the electronic supplementary material (ESM). EH conducted the seed feeding experiments with rodents and partly wrote the corresponding section. KA performed the field seed removal experiment and some of the experiments in the ESM. SS contributed to the germination experiment and to experiments in the ESM. MG assisted in the statistical analysis and gave comments on the manuscript. WW was involved in the study design, the analysis and manuscript improvement.

CHAPTER THREE

The Seed Dispersal Potential of Some Gastropod Species and Indications for Predator-Avoidance of Slug-Consumed Seeds

Manfred Türke • Wolfgang W. Weisser

Manuscript status: submitted to *PLoS ONE* (25.02.2011)

To test the generality of the finding that gastropods are feeding on seeds of myrmecochores and to assess their dispersal potential, I conducted seed feeding experiments in the laboratory with four gastropod species (and two age-classes of one species) and seven myrmecochores. To test whether animals in the field are less likely to remove seeds of which slugs had consumed the elaiosomes or seeds which had been defecated, I conducted a field seed removal experiment.
I designed and conducted the experiments, collected seeds and gastropods, analyzed the data and wrote the manuscript. WW helped to improve the study design and the manuscript.

CHAPTER FOUR

The Influence of Body Mass on the Seed Dispersal Potential of Slugs

Manfred Türke • Wolfgang W. Weisser

Manuscript status: submitted to *Integrative Zoology* (10.03.2011)

We found that juvenile individuals of red slugs (*Arion rufus*) did not swallow large seeds, while mature individuals did. However, there was a great divergence between individuals in the proportion of seeds swallowed. We tested, whether the proportion of seeds swallowed was correlated with the body mass of mature red slugs and we applied the results to natural populations with different body mass distributions.
I designed and conducted the experiment, analyzed the data and wrote the manuscript. WW helped to improve the manuscript.

CHAPTER FIVE

Are Gastropods, rather than Ants, Important Seed Dispersers of Myrmecochorous Forest Herbs?

Manfred Türke • Kerstin Andreas • Martin M. Gossner • Esther Kowalski • Markus Lange • Steffen Boch • Stephanie Socher • Jörg Müller • Daniel Prati • Markus Fischer • Rainer Meyhöfer • Wolfgang W. Weisser

Manuscript status: submitted to *The American Naturalist* (11.02.2011) as a Natural History Miscellany Note

We compared ant, gastropod and myrmecochore abundances in beech forests in a large scale approach and found a negative relationship of myrmecochores and ants and a positive relationship of myrmecochores and gastropods. We further conducted a seed removal experiment in a large scale approach and found a high contribution of gastropods to seed removal. Video recordings were used to evaluate the influence of depots with restricted access to certain animal taxa on arthropods.

I designed, contributed partly to and supervised the field seed removal experiment, conducted the video recording observations, analyzed all data and wrote the first draft of the manuscript. KA conducted the field seed removal experiment. MG, EK, ML and I gathered the ant and gastropod data by pitfall trap samples. SB, SS, JM, DP and MF provided the vegetation data. RM helped in the video recording observations. WW was involved in study design. All authors gave comments on the manuscript.

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Prof. Dr. Wolfgang W. Weisser

CHAPTER TWO

Seed Consumption and Dispersal of Ant-Dispersed Plants by Slugs

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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Seed consumption and dispersal of ant-dispersed plants by slugs

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Abstract In beech-dominated forests in Central Europe, many spring geophytes show adaptations to seed dispersal by ants (myrmecochory). Ants, however, can be rare in such moist forests. Motivated by observations of slug feeding on seeds we investigated the seed consumption of two plant species, *Anemone nemorosa* and *Asarum europaeum*, by slugs, in a series of experiments. In a seed predation experiment in a beech forest, we found that seed removal was strongly reduced when gastropods were excluded from the seed depots. The contribution of insects, including ants, and rodents to seed removal was relatively less but differed between May and July. In the laboratory, slug species, in particular *Arion* sp., consumed seeds of both plant species. Slugs either consumed the elaiosomes of seeds or swallowed seeds intact. Swallowed seeds were defecated undamaged and germinated as well as control seeds when buried overwinter, indicating the potential for seed dispersal by slugs. We also recovered seeds of myrmecochores in the faeces of several slugs caught in forests. In a slug release experiment in the forest, slugs moved up to 14.6 m (mean 4.4 m) in 15 h, which is the median gut passage time of seeds based on measurements made in the laboratory. We also found that when slug-defecated seeds were offered to rodents, these were less attractive than control seeds, suggesting that passage through the slug gut reduces seed predation risk. Our results demonstrate that slugs are significant consumers of elaiosomes or entire seeds of ant-dispersed plants and that they can function as seed dispersers of these plants.

Keywords Arion · Beech forest · Gastropodochory · Granivores · Myrmecochores

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Introduction

Adaptations of understory herbs to seed dispersal by ants is a common phenomenon in European and North American temperate deciduous forests where myrmecochores can represent up to 30–50% of herbaceous biomass (Sernander 1906; Handel et al. 1981; Gorb and Gorb 2003; Seifert 2007). The diversity of ant-dispersed plants is high in beech-dominated forests of Germany, with up to 45 plant species considered to be myrmecochores (Sernander 1906; Ulbrich 1939). In the Hainich National Park, an unmanaged beech forest in Central Germany, 29% of the herbaceous plant species are myrmecochores, which accounts for 71% of the herbaceous plant cover (S. Boch et al., unpublished data). Seeds of myrmecochorous plants regularly bear an elaiosome, a lipid-rich appendage (Bresinsky 1963), which is consumed by ants and acts as a nutritional reward (Edwards et al. 2006; Fokuhl et al. 2007; Fischer et al. 2008). Benefits from seed dispersal by ants include a reduction of intra- and interspecific competition, translocation of seeds to the nutrient-rich environment of an ant nest, and a reduction in seed predation (summarized in

Gorb and Gorb 2003 and in Giladi 2006). In cool and moist forests, such as Central European beech forests, ant diversity and abundance can, however, be very low (Seifert 1986, 2007; Weseloh 1995; Retana and Cerda 2000; Anu and Sabu 2007) such that dispersal by ants is likely to be rare, raising the questions of if and how seeds of myrmecochores are dispersed.

The seed predation rate of understorey herbs in temperate deciduous forests varies dramatically, with reported predation values ranging from almost zero (Smith et al. 1989; Ruhren and Dudash 1996) up to 97.9% (Culver and Beattie 1978; Heithaus 1981; Turnbull and Culver 1983; Kjellsson 1985a; Smith et al. 1986; Muir 1997; Anderson and MacMahon 2001). The most prominent seed predators in forests are rodents (Janzen 1971; Heithaus 1981) and arthropods, in particular, carabid beetles, although many insects have been shown to prey on plant seeds (Cardina et al. 1996; Ohkawara et al. 1996; Honek et al. 2003; Hurst and Doberski 2003; O'Rourke et al. 2006; White et al. 2007). One group of seed predators that has so far received little attention is the gastropods even though slugs and snails have been reported to prey on seeds (Cardina et al. 1996; Kollmann and Bassin 2001; Hurst and Doberski 2003; Simonetti et al. 2003). Elaiosomes on seeds of myrmecochorous plants have a dual role: on the one hand, they attract ants, the natural seed dispersers; on the other hand, elaiosomes may also attract a diverse invertebrate fauna, including insects (Ohkawara et al. 1996), slugs, and snails (Mesler and Lu 1983; Muir 1997), which feed on the elaiosomes and may damage seeds. Our knowledge of the importance of gastropods in terms of seed predation, in particular the predation of entire seeds and elaiosomes of myrmecochores is, however, still very limited because most observations of slug seed predation to date have been made incidentally.

There is a diverse fauna of gastropods, in particular slugs and snails, in temperate beech forests (Bless 1977; Kerney et al. 1983; Bogon 1990; Müller et al. 2005). During preliminary surveys in beech forests of Central Germany, on several occasions, we observed slugs feeding on the seeds of myrmecochores; in contrast, ants were rarely observed. Some slugs appeared to swallow entire seeds, which raised at least the possibility of seed dispersal through slug consumption. We therefore conducted a series of laboratory and field experiments to address the following questions: (1) How high is the seed predation rate of myrmecochorous plants in beech forests and what taxa contribute most to seed removal and elaiosome consumption? (2) What is the fate of seeds when they are offered to common forest slugs? (3) Is there evidence for slugs transporting seeds under natural conditions? (4) If slugs transport seeds, how far are they transported and what is their germination rate after defaecation? (5) How does seed consumption by slugs interact with seed predation by rodents?

Materials and methods

Sampling area

Sampling, field experiments and preliminary surveys took place in the Hainich region south of Mühlhausen in Thuringia, Germany, at 400–500 m a.s.l. (10°27′E, 51°05′N). The main features of the landscape are deciduous forests dominated by beech (*Fagus sylvatica*). There are varying forest management intensities, including intensive age-class forests and unmanaged beech forest (Hainich National Park). The Hainich National Park forest is an old-growth, unevenly aged (0–250 years) forest and has been unmanaged for about 50 years. It includes pure and mixed beech stands (*Fagus sylvatica*, *Fraxinus excelsior*, *Acer pseudoplatanus*, *Carpinus betulus*, *Tilia* sp., *Acer campestre*, among others) with a multi-layered canopy dominated by old and tall trees and natural gaps. In contrast, in the age-class forest, there are almost pure stands of *Fagus sylvatica* consisting of individuals of similar age. Our study is part of the biodiversity exploratories project, and more detailed information on the sampling area can be obtained from the project's website (http://www.biodiversity-exploratories.de/). For plot numbers of each field experiment, see Table S1 in the Supplementary Electronic Material (ESM).

Plant material

Diaspores of two myrmecochorous spring geophytes, *Anemone nemorosa* L. (wood anemone; Ranunculaceae) and *Asarum europaeum* L. (European wild ginger; Aristolochiaceae) (Sernander 1906; Ulbrich 1939; Gorb and Gorb 2003), were collected in the Hainich region and those of *A. europaeum* were also collected in deciduous forests surrounding Jena, Thuringia (coordinates 11°36E, 50°56′N), where they were available in great numbers, just before seed ripening (April to June). All seeds were kept in a freezer at −20°C until used in the experiments. Achenes of *A. nemorosa* (called seeds in the following text) measure 4.2–5 × 1.8–2.4 mm, and seeds of *A. europaeum* measure 3.6–4.4 × 2.1–2.4 mm (Bojnansky and Fargasova 2007). Seeds of both plants bear a distinct elaiosome (Sernander 1906; Canullo 1985). *A. nemorosa* is common in the Hainich region and has a low attractiveness to ants (Sernander 1906); *A. europaeum* is less common and is attractive to ants (Gorb and Gorb 2003). When offered to ants simultaneously, significantly more seeds of *A. europaeum* were removed than seeds of *A. nemorosa* (see ESM Appendix S2).

Animal material

Slugs

All slugs used in the experiments were collected in deciduous forests in the Hainich region and kept in the climate chamber at 20°C. Slug species were identified according to Ehrmann (1956), Kerney et al. (1983), Bogon (1990), Noble (1992) and Zeissler (1998).

Based on the observation data of a preliminary seed predation experiment (see ESM Appendix S3), the main study species were *Arion* (Arion) *rufus* L. (red slug) and *Limax* (Limax) *cinereo-niger* Wolf (ash-grey slug). Both species are almost equal in size, measuring between 10 and 20 cm and both are quite abundant in beech forests (Ehrmann 1956; Kerney et al. 1983; Bogon 1990; Müller et al. 2005). All individuals of both slug species were >10 cm when stretched and considered to be adult.

We also conducted experiments with medium-sized *Arion* individuals (<80 mm body length) of different species. Medium-sized slugs (length 50 ± 4 mm, $n = 18$) were killed and identified after completion of the experiments; these included 13 juvenile *A. rufus*, two *Arion* (Mesarion) *subfuscus* Draparnaud and three individuals that could not be identified clearly, possibly *Arion* (Carinarion) *silvaticus* Lohmander, *Arion* (Carinarion) *fasciatus* Nilsson or *Arion* (Carinarion) *circumscriptus* Johnston.

Slugs >5 cm in body length were kept in fauna boxes measuring 27 (length) × 18 (width) × 17 (height) cm (Savic Fauna box, 6 L; http://www.savic.be). Slugs of <5 cm in body length were kept in 9-cm petri dishes. The slugs were fed once a week with lettuce, carrot and wild herbs. All food was removed 1 day before an experiment started. The slugs were kept on wet paper towels which they fed on frequently.

Rodents

We chose *Apodemus flavicollis* Melchior (yellow-necked mouse) and *Myodes glareolus* Schreber (bank vole) as our target species as those rodent species were captured in our preliminary rodent abundance survey in the Hainich National Park (see ESM Appendix S4). While *M. glareolus* is mainly graminivorous, *A. flavicollis* prefers the seeds of woody plants (Corbet and Ovenden 1980). Individuals were trapped in the surrounding of Remderoda field station near the University of Jena (coordinates 11°31′E, 50°56′N) with Ugglan multiple live capture traps. Animals were kept singly in plastic fauna boxes [40 (length) × 25 (width) × 15 cm (height)] closed at the top with a metal grid. Boxes were provided with sawdust and paper towels. For each individual, the basic amount of food per day was 5 g compressed mouse pellets (containing different types of grain), 5 g fresh apple pieces and fresh water (not limited in a drop-bottle).

Seed predation by different taxa in the field

To assess seed predation of myrmecochores in a natural habitat and to evaluate the contribution of different taxa (rodents, gastropods and insects) to seed removal and elaiosome consumption (our question 1), we conducted a seed predation experiment in five forest plots in the Hainich National Park with a minimum distance of 200 m between plots (see ESM Table S1) in which we offered seeds of *A. nemorosa* and *A. europaeum* at the time of their ripeness, namely, on 25 May 2009 and 10/11 July 2009, respectively. The seeds were offered in groups of ten on a 10 × 10-cm wooden plate (seed depot) that was protected against rain by a 12.5 × 12.5-cm plastic roof.

There were four predator exclusion treatments. Treatment A was the control, in which seeds were not protected against predators, and treatment B was rodent exclusion, in which seed depots were covered by a 11 × 11 × 4-cm metal cage with a mesh size of 28 × 10 mm (rhombus-shaped mesh) that excluded rodents but allowed gastropods and insects to enter the seed depot. The third treatment, treatment C, was that of gastropod exclusion, in which slug-deterring copper wire (SNA.P Snail Protect, Gartenring, Germany) was attached to the wooden plate and covered with slug repellent (Schneckenabwehrpaste, IRKA); 18-cm-long bridges of zinc-coated, 2.8-mm strong wire extended in four directions from the seed depot, with one end dug into the forest floor and the other end leading to the centre of the seed depot. It was therefore possible for ants (and rodents) to cross the repellent and to enter the seed depot, but gastropods were deterred. The fourth treatment, treatment D, was both gastropod and rodent exclusion; the set-up resembled that of treatment C, but the seed depot was additionally covered by a cage and ant bridges led through the cage mesh, allowing only insects to enter the seed depot. The different treatment seed depots were separated by 4 m.

The effectiveness of the predator exclusion techniques was tested in preliminary experiments (ESM Appendices S5–S8). The cages were found to successfully restrict rodents from entering the seed depots, but they did not prevent large slugs from entering the cages (see ESM Appendix S5). The combination of copper wire and slug repellent significantly reduced slug entrance (ESM Appendix S6). The slug repellent also deterred ants (ESM Appendix S7), but the ant bridges allowed ants to enter a seed depot protected by slug repellent (see ESM Appendix S8). Slug repellent did not deter rodents (ESM Appendix S9).

Seed depots were checked after 3 days, and the remaining seeds with or without elaiosome were scored. For *A. europaeum*, the consumption of elaiosomes was assessed in the field, while seeds of *A. nemorosa* were transported to the laboratory and examined under a microscope for feeding traces on the seed coat and the peduncle. There were two replicates of each group of four seed depots in each 100 × 100-m plot, installed in two corners of the plot. Thus, in total we offered five plots × two plant species × two replicates × four treatments × 10 seeds per seed depot = 800 seeds.

We calculated the number of seeds removed by each taxon as follows: (1) total removal = the number of seeds removed by all predators in treatment A (control); (2) removal by gastropods = seeds removed in treatment B − seeds removed in treatment D; (3) removal by rodents = seeds removed in treatment C − seeds removed in treatment D; (4) removal by insects = seeds removed in treatment D. Treatment means were calculated as the average of both replicates per plot. Thus, analyses were based on five replicates (plots).

We calculated the contribution of a particular taxon to total seed removal (and to seeds with elaiosome damage of remaining seeds) by dividing the number of seeds removed (seeds with elaiosome damage) by a particular taxon by the total number of seeds removed (seeds with elaiosome damage) by all predators in the plot. Note that this could result in values exceeding 100% either because of compensatory feeding of taxa other than the excluded one or due to a higher attractiveness of seeds in a certain treatment.

The fate of seeds when offered to slugs

In two experiments we tested if slugs feed on entire seeds or elaiosomes of *A. nemorosa* or *A. europaeum* and how long consumed seeds remain in the gut of a slug (question 2).

Slug seed feeding experiment

Individual slugs of *A. rufus*, *L. cinereo-niger* and *Arion* spec. (<80 mm) were offered six seeds of a plant species at a time; only five seeds of *A. europaeum* were offered to *A. rufus*. On average, seeds of a plant species were offered to 20 ± 2 individuals (mean ± standard error) of a species.

The seeds were exposed to slug consumption for 48 h, and the outcome was classified as follows: (1) swallowing of the whole seed, (2) consumption of elaiosome only, and (3) no consumption. The numbers of offered seeds of a plant species that were swallowed or of which only elaiosomes were consumed per individual were used for the statistical analysis. Some individuals were offered seeds of both plant species, and we accounted for reuse of individuals in the analysis. When seeds were apparently undamaged, they were examined under the microscope at 65× magnification. Faeces were collected after 24 and 48 h and examined for digested and defecated seeds. Defecated seeds were also examined under the microscope.

Gut passage times for seeds of A. europaeum

We determined gut passage times of *A. europaeum* seeds using *A. rufus* as a model species. *A. europaeum* seeds were used because we expected that a high proportion of these seeds would be swallowed by slugs, as indicated by the slug seed feeding experiment. Ten slugs were allowed to feed on ten seeds each on 12 July 2008 from 10.00 a.m. to 11.00 a.m. in fauna boxes; after 11:00 a.m., all unswallowed seeds were removed. The boxes were checked for faeces hourly for the next 33 h. All faeces were collected and microscopically examined for defecated seeds immediately after each check. The number of defecated seeds was recorded. Seeds retrieved from faeces were examined under the microscope, and the proportion of the elaiosome that was still remaining was estimated as 0, 25, 50, 75 or 100%. At the beginning of the experiment, when the remaining seeds were removed, and after 6 h, slugs were allowed to feed ad libitum on a carrot (*Daucus carota*; approx. 0.5 g fresh weight) and a dandelion (*Taraxacum officinale*; approx. 0.5 g). Wet paper towels as an additional food source were available throughout the experiment.

Natural seed dispersal by slugs

To test if slugs also swallow and disperse seeds under natural conditions (question 3), slugs were caught in forests in the Hainich region (see ESM Table S1) and kept individually in fauna boxes for 48 h. Faeces were collected and examined for seeds using a microscope. The first group of *A. rufus* individuals ($n = 22$) was collected on 29 May 2008, when the seeds of *A. nemorosa* were ripe and plants had already shed some of their seeds. The second group of *A. rufus* individuals ($n = 23$) was collected on 2 June 2008, when most of the seeds of *A. nemorosa* had been shed. The conditions were dry on both of these dates, and slugs were mainly encountered among coarse woody debris. An additional 264 individuals of *A. rufus* were collected at seven different forest sites (mean $n = 38$ individuals per site, range 17–61) on 19 and 20 May 2009. Approximately one-third to one-half of the seeds of *A. nemorosa* was shed on these dates, although number of seed-bearing plants and proportion of shed seeds varied between sites. Wet weather conditions prevailed, and slugs were active on these dates. Only slugs found within 20 m of *A. nemorosa* plants were collected.

Seeds from faeces were identified by comparisons to collected seed material and by using the guidelines of Bojnansky and Fargasova (2007).

Slug dispersal distances and seed germination after defaecation

In two experiments we tested how far slugs would theoretically transport seeds and whether seeds would be still viable after defaecation (question 4).

Slug dispersal experiment

To assess the dispersal distances of *A. rufus* in a natural habitat, we conducted a dispersal experiment in forests in the Hainich (see ESM Table S1). One replicate of the dispersal experiment was conducted in the Hainich National Park (15 July 2007) and two were conducted in two different 20- to 40-year-old stands of the age-class forest (11 July 2007 and 22 August 2007). A 40 × 40-m plot was marked in the forest, and all individuals of *A. rufus* that were found on the ground were collected. Twenty medium to large-sized individuals of *A. rufus* were marked by skirt clips in which a small triangle is cut out on the right side of the distal part of the skirt (foot fringe). This newly established method is suitable for a very short-term marking of slugs. Other methods commonly used for marking slugs (Grimm 1996 and references therein) also require injuring the slugs. After the skirt had been clipped, the slugs were allowed at least 30 min to recover before being released.

The slugs were released together at 6.00 p.m. at a marked position in the centre of the plot. Slugs are mainly active at night (e.g. Hommay et al. 1998; Grimm and Schaumberger 2002), but they are also active during the daytime in moist forests with a closed canopy (personal observations). In the following morning, 14 h after release, the 40 × 40-m plot was searched intensively for the marked slugs. For each encountered individual, the distance from and the direction to the release point (compass) was measured. Dead wood was turned over to search for hidden slugs within the 20 × 20-m centre of the plot.

Data from the gut passage times experiment were used to calculate potential dispersal distances of seeds when transported by slugs.

Germination experiment

This experiment tested whether seeds were still germinable after being passed through the gut of the slug using *A. nemorosa* and *A. rufus* as a model system. Seeds were collected on 02 June 2008 and kept in the fridge at 4°C until fed to slugs or planted as control seeds on four dates between 09 June 2008 and 05 August 2009. Twenty seeds were exposed on petri dishes to individuals of *A. rufus*, kept singly in fauna boxes ($n = 46$). After 48 h all seeds were collected. Defecated seeds ($n = 254$) and seeds with feeding marks on the seed coat or peduncle ($n = 109$) were planted immediately 2 cm deep into potting soil in flower pots [10 (diameter) × 7.5 (height) cm]. No more than five seeds (mean 4.7) of only one type were planted per flower pot. For each pot with treatment seeds, one pot with five control seeds was established on the same day ($n = 380$ control seeds). All flower pots were watered regularly and kept in the climate chamber at 20°C until they were transferred to the forest on 07 August 2008. Flower pots were distributed on three plots in the Hainich National Park, dug into the soil and covered with leaf litter afterwards to simulate natural conditions for germination. A flower pot with control seeds was arranged next to each pot with treatment seeds. There were a total of 76 pots with control seeds, 24 pots with seeds with feeding marks and 53 pots with defecated seeds. The pots were covered with 2-mm wire mesh to exclude seed predators. The pots were controlled twice for germinated seeds (indicated by the presence of a radicle)—10 February 2009 and 02 March 2009. Germinated seeds were counted and removed from the pots. The germination experiment was terminated after the second check as the pots could not be 100% protected against seed predators after the first opening.

Rodent feeding behaviour of slug-consumed seeds

We also tested if prior consumption by slugs affected seed attractiveness to rodents (question 4). Seeds were offered on a wooden plate (height 3 cm). In the first 2 h of the experiment, observations were made every 5 min, and the number of remaining seeds was noted. After 2 h, the inspections took place every 15 min until the experiment was ended. Using the collected data, we calculated consumption time as a measure of the preference for a particular type of seed. Individuals were reused for different experiments.

In Experiment (1), two untreated seeds of *A. europaeum* (control seeds) and two seeds of *A. europaeum* previously digested by slugs (*A. rufus*) (treatment seeds) were offered simultaneously either to *M. glareolus* ($n = 11$) or to *A. flavicollis* ($n = 9$). The experiment was carried out on 18 June 2007 and lasted 160 min.

Experiment (2) was similar to experiment (1), but treatment seeds had only their elaiosomes eaten by slugs (*M. glareolus*, $n = 9$; *A. flavicollis*, $n = 7$). The experiment took place on 19 June 2007 and lasted for 290 min.

After an experiment had ended, the seeds remained in the cage and another check was made on the following morning. All seeds were eaten by this time. For seeds eaten

after the end of the experiment, we noted the time of the last check on the previous day as the time of consumption. Faeces samples of all animals were collected after the experiment and examined microscopically for the remains of digested seeds.

Statistical analyses

Statistical analyses were performed with SPSS ver. 15.0.1 (SPSS, Chicago, IL). Analyses of variance (ANOVAs) for the seed predation and rodent feeding experiments and the analysis of the slug seed feeding experiment were performed using R 2.8.0 (http://www.r-project.org/). In the slug seed feeding experiment, we compared the number of swallowed seeds (response is the number of seeds being not swallowed) and seeds with consumed elaiosomes (response is the sum of seeds not consumed and of seeds swallowed) between gastropod species and plant species in a linear mixed-effects model (fit by the Laplace approximation for binomial errors) using the function "lmer" in the lme4 package. Plant species and slug species were treated as fixed effects and slug individuals as a random effect. Multiple comparisons of means (Tukey contrasts) were performed using the function "glht" in the multcomp package. All data were tested for normality and transformed where possible, otherwise non-parametric tests were used. Means and standard errors of variables are given throughout.

Results

Seed predation by different taxa in the field

The predator exclusion treatments generally worked well. One depot (rodent exclusion) in the *A. nemorosa* experiment and one depot (gastropod exclusion) in the *A. europaeum* experiment had to be excluded because they were destroyed by animals, most likely a wild boar. Mucous trails were found on seven of the 40 depots applied with slug repellent (gastropod exclusion, gastropod + rodent exclusion), and these depots were also excluded from the analysis, resulting in one plot that could not be used for calculating the contributions of different taxa to seed consumption and removal in the *A. nemorosa* experiment.

Of the 340 *A. nemorosa* seeds exposed in the remaining seed depots, a total of 112 (32.9%) were removed and a further 80 seeds (23.5%) showed signs of feeding on the elaiosomes. Of the 350 *A. europaeum* seeds exposed in the seed depots, 133 (38%) were removed and in a further 73 seeds (20.9%) the elaiosomes had been consumed. Seed cores of seeds which remained in the seed depots were all intact, with the exception of five *A. nemorosa* seeds; these seeds were treated as being removed from seed depots.

During the observations, there was ample evidence of gastropod interest in the seed depots: three individuals of *A. rufus*, one individual of *L. cinereo-niger* and seven snails were found on the control and rodent exclusion depots in the *A. europaeum* experiment. Mucous trails of slugs or snails were found on 53% of the control and rodent exclusion depots in the *A. nemorosa* experiment and on 70% of the depots in the *A. europaeum* experiment.

In subsequent analyses, we use the term seed consumption for the sum of removed seeds and seeds which had their elaiosomes consumed. The total number of seeds that were consumed was higher in the control and rodent exclusion treatments than in the gastropod or gastropod + rodent exclusion treatments (Figs. 1a, b; $F_{3,30} = 25.32$, $p < 0.001$). There was no significant difference between the plant species ($F_{1,30} = 0.77$, $p = 0.39$). Elaiosomes in *A. europaeum* were only damaged in the controls and in the rodent exclusion treatments, while seeds with feeding traces were found in all treatments involving *A. nemorosa*. There was, therefore, a significant interaction between plant species and exclusion treatment ($F_{3,30} = 4.45$, $p = 0.012$). The total number of seeds removed also differed between treatments, with most seeds being removed from the controls and rodent exclusion treatments (Figs. 1a, b; $F_{3,30} = 8.55$, $p < 0.001$), but there was again no difference between the two plant species ($F_{1,30} = 0.0053$, $p = 0.94$).

The contribution of different taxa to seed removal and to seed consumption differed between the May and July tests and hence between *A. nemorosa* and *A. europaeum* (Figs. 1c, d). While gastropods were responsible for a high proportion of seed removal in both plant species, rodents had a minor impact ($-34 \pm 65\%$) and insects a stronger contribution ($43 \pm 39\%$) to seed removal of *A. nemorosa* seeds in May. With respect to the removal of *A. europaeum* seeds in July, the contribution of insects was only marginal ($1 \pm 1\%$), while rodents now contributed $51 \pm 46\%$ to seed removal. Insects contributed strongly to the consumption of *A. nemorosa* seeds ($52 \pm 22\%$) but very little to that of *A. europaeum* seeds ($1 \pm 1\%$) for which consumption could mostly be attributed to gastropods ($106 \pm 7\%$).

The fate of seeds when offered to slugs

Slug seed feeding experiment

All slug species fed on seeds of both plant species (Fig. 2). Seeds offered were either swallowed or remained unswallowed. The elaiosomes of unswallowed seeds were examined for feeding traces.

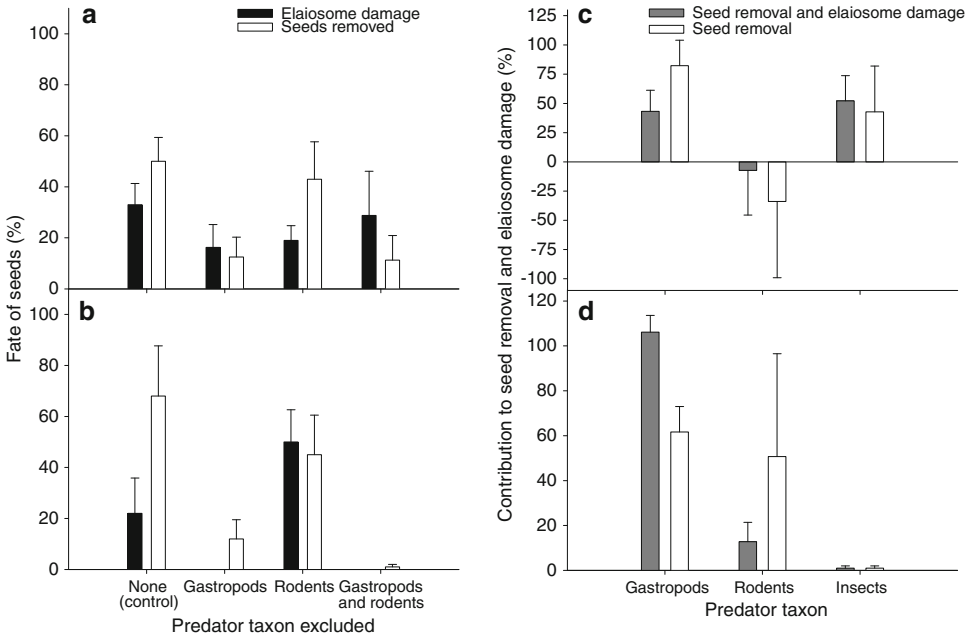

Fig. 1 Fate of exposed seeds of *Anemone nemorosa* (**a**) and *Asarum europaeum* (**b**) in the field seed predation experiment ($n = 5$ plots) and the contribution of different taxa to seed removal and elaiosome damage and to seed removal only of *A. nemorosa* (**c**) ($n = 4$ plots) and *A. europaeum* (**d**) ($n = 5$ plots). Results are given as the mean + standard error (SE)

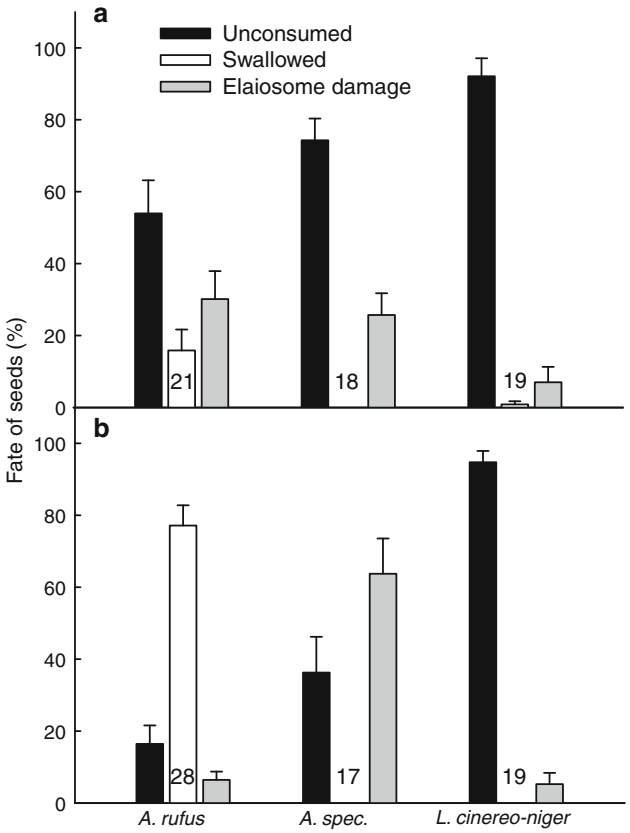

Fig. 2 Fate of seeds of *A. nemorosa* (**a**) and *A. europaeum* (**b**) when offered to slugs in the laboratory. Seeds were either swallowed, suffered feeding damage to their elaiosomes or remained unconsumed. The *numbers* indicate the number of slugs to which seeds of each plant species were fed. Results are given as the mean + SE

We use the term seed consumption to indicate the sum of swallowed seeds and of seeds which had their elaiosomes consumed in the following treatments. Elaiosomes of *A. europaeum* seeds were often consumed entirely by slugs while only the peduncle (location of the elaiosome; Canullo 1985) and/or parts of the seed coat or the entire seed coat of *A. nemorosa* seeds were consumed (all feeding was treated as elaiosome damage in the following discussion). Seeds swallowed by slugs showed no signs of a destroyed seed core when they were collected from the faeces and examined. *A. europaeum* elaiosomes were also mostly undamaged after gut passage, while feeding traces were often found on seeds of *A. nemorosa.*

More seeds of *A. europaeum* than of *A. nemorosa* were swallowed by slugs (Fig. 2; GLMM $z = 5.93$, $p < 0.001$) and showed elaiosome damage ($z = 4.28$, $p < 0.001$). Almost half of the seeds of *A. nemorosa* exposed to *Arion rufus* were consumed (46%), 25% were consumed by medium-sized slugs of *Arion* spec. (<80 mm) and only 8% were consumed by *Limax cinereo-niger*. Seed consumption of *A. europaeum* by *Arion* species was high and ranged between 64 and 84%. In contrast, *L. cinereo-niger* consumed only a few seeds (5%). *A. rufus* swallowed entire seeds of both plant species but consumed many more seeds of *A. europaeum* (77%) than of *A. nemorosa* (15%). Just one individual of *L. cinereo-niger* swallowed a single seed of *A. nemorosa*. Medium-sized slugs did not swallow seeds but only consumed the elaiosomes. However, only between *L. cinereo-niger* and *A. rufus* were significant differences found in the proportion of swallowed seeds ($z = -4.52$, $p < 0.001$). Medium-sized slugs consumed more elaiosomes of seeds than the two large slug species *A. rufus*

($z = 0.73$, $p < 0.001$) and *L. cinereo-niger* ($z = 4.32$, $p < 0.001$).

Gut passage times for seeds of A. europaeum

A total of 63 of the 100 seeds offered were swallowed by the ten slugs (range 1–10 seeds per slug, mean 6 ± 1 seeds). The first seeds were defecated 11 h after being swallowed, and the last seeds were recovered after 33 h (Fig. 3). Median gut passage time was 15 h. Approximately 60% of the defecated seeds still retained the entire elaiosome, and average elaiosome damage of all swallowed seeds was 25 ± 5% of elaiosome volume. The remains of elaiosomes were found regularly in the faeces, suggesting that the elaiosome was damaged while the faeces were being searched for seeds or during the passage of the seeds through the guts of the slug. The degree of damage of elaiosomes was not correlated with gut passage time (Spearman's rank correlation; $n = 63$; $R = 0.14$; $p = 0.26$). To summarize, not only were the seeds undamaged by gut passage but the elaiosomes also did not suffer greatly from ingestion by slugs.

Natural seed dispersal by slugs

A total of 19 seeds were found in the faeces of 11 of 309 individuals of *A. rufus* (3.6% individuals with seeds, range 0–9% depending on forest site). One to eight seeds were found in faeces of one individual (1.7 ± 0.6 seeds per individual). Seed cores of only two seeds were destroyed, while the other seeds were all intact. Sixteen seeds were identified as seeds of *Anemone nemorosa*, one as a seed of *A. ranunculoides* and two seeds belonged most likely to myrmecochorous sedges of the genus *Carex* (Cyperaceae) which can commonly be found in forests in the Hainich region.

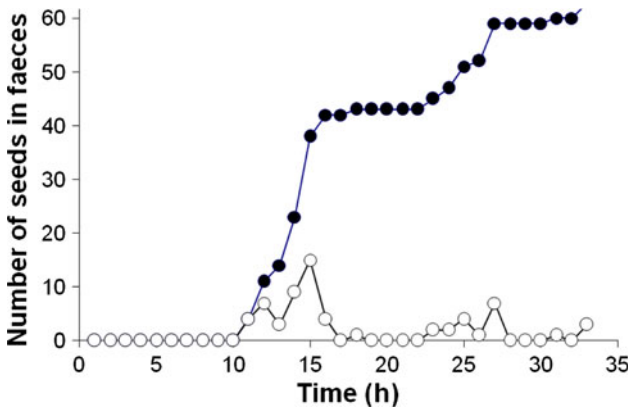

Fig. 3 Gut passage time of *A. europaeum* seeds after ingestion by the red slug *A. rufus*. *Open circle* Number of seeds found in faeces at a particular time after ingestion, *filled circle* cumulative number of defecated seeds ($n = 63$ seeds)

Slug dispersal distances and seed germination after defaecation

Slug dispersal experiment

Of the 60 marked and released slugs, 54 were found again, six of which were found underneath dead wood. The average distance to the release point in all three replicates was 4.42 ± 0.48 m (range 0–14.6 m). There was no preferential dispersal into a particular direction in any of the three replicates (chi-square tests and circular statistics, Monte Carlo simulations with 10,000 steps; $p > 0.05$) as well as no significant differences in the distances from the position where slugs were found to the release point between directions (Kruskal–Wallis H tests; $p > 0.05$).

Germination experiment

A total of 33% of all seeds had germinated on 10 February 2009 (first check) and 42% had germinated 02 March 2009 (second check). Of the germinated seeds on 10 February, 86 were defecated seeds (34%), 37 seeds which had traces of feeding (34%) and 121 were control seeds (32%); on 02 March 2009, the proportions were 41, 43 and 42%, respectively. There was no difference in the germination rate between any treatment on both dates when total numbers of germinated seeds were compared (chi-square tests; $p > 0.05$). There was also no difference in the proportions of seeds that had germinated per flower pot on 10 February 2009 when pots of control seeds were compared to their neighbouring pot with respect to seeds with feeding marks (Wilcoxon signed ranks test, $n = 24$, $Z = -0.24$, $p = 0.81$) or defecated seeds ($n = 54$, $Z = -0.05$, $p = 0.96$). There were still no differences on 02 March 2009 in the proportions of germinated seeds of control seeds compared to neighbouring seeds with feeding marks ($n = 24$, $Z = -0.68$, $p = 0.50$) or defecated seeds ($n = 54$, $Z = -0.51$, $p = 0.61$).

Rodent feeding behaviour of slug-consumed seeds

All seeds offered to the individuals of *M. glareolus* and *A. flavicollis* were consumed completely, irrespective of treatment, with 97% of all seeds consumed within the first 5 h and all seeds completely consumed on the last check after 12 h. Only seed fragments were found in the faeces of the rodents, suggesting that rodents always destroy consumed seeds.

When seeds of *A. europaeum* from slug faeces were offered to rodents along with control seeds, control seeds were consumed faster than seeds from slug faeces (Fig. 4; split-plot ANOVA, $F_{1,19} = 6.74$, $p = 0.017$). Yellow-necked mice *A. flavicollis* took more time to consume control

and treatment seeds (109 ± 13 min) than individuals of *M. glareolus* (40 ± 7 min; $F_{1,19} = 17.53$, $p < 0.001$).

There was no difference in time to consumption between seeds with their elaiosomes previously eaten by slugs and control seeds (Fig. 4; split-plot ANOVA, $F_{1,16} = 0.01$, $p = 0.97$). Yellow-necked mice *A. flavicollis* again took more time to consume seeds (159 ± 33 min) than individuals of *M. glareolus* (55 ± 15 min; $F_{1,15} = 6.10$, $p = 0.026$).

Discussion

The aim of our study was to investigate the predation and dispersal of seeds of supposedly ant-dispersed plant species in temperate beech forests. The main result of our study is that large slugs, in particular *Arion rufus*, in addition to insects and rodents, may play an important role in terms of elaiosome predation and seed removal. In laboratory experiments, slugs readily consumed seeds of two myrmecochorous plant species, either by eating the elaiosome or by swallowing seeds entirely. Seeds swallowed by slugs were undamaged after passage through the gut, in contrast to consumption by rodents which destroyed the seeds.

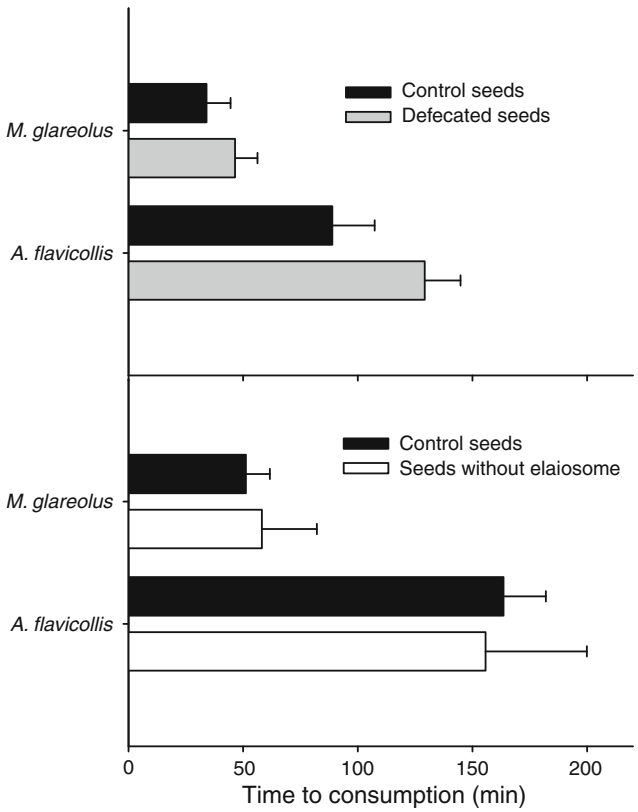

Fig. 4 Effect of slug feeding on the consumption of *A. europaeum* seeds by the rodents *M. glareolus* and *A. flavicollis*. *Top* Seeds defecated by slugs (*M. glareolus*: $n = 11$; *A. flavicollis*: $n = 9$). *Bottom* Seeds without elaiosome (*M. glareolus*: $n = 9$; *A. flavicollis*: $n = 7$). Results are present as the mean + SE

Thus, slugs may act not only as predators of elaiosomes but also as seed dispersers. Interestingly, our results also suggest that dispersal of seeds by slugs may influence seed survival, not only because of the distances moved but also because of the potential to reduce predation by rodents.

Seed consumption by different animal taxa

In our field experiment, gastropods were responsible for most of the damage inflicted on the elaiosomes and were also the most important taxon in terms of seed removal. Overall seed removal from seed depots did not differ between May and July and, consequently, also not between the two plant species. When we offered seeds of *A. europaeum*, insects were responsible for almost half of the seed removal, while rodents had no influence on seed removal for *A. nemorosa* in May, while the reverse was true in July. This change could be related to different attractiveness features of seeds of the two plant species or to different activity peaks of the predator taxa. In our slug seed feeding experiment, more seeds of *A. europaeum* than of *A. nemorosa* were swallowed by slugs. Sernander (1906) reported that the seeds of *A. nemorosa* had a low attractiveness to ants, while seeds of *A. europaeum* have been reported to be very attractive to ants (Gorb and Gorb 2003), which was supported by the results of our ant seed preference experiment (see ESM Appendix S2). These findings are in contrast to what we observed in our insect treatment. Ants were rare in the mature beech forests (see ESM Appendix S10), thus our insect category most likely includes seed predators, such as carabid beetles. While small insects (small Dermaptera, Collembola) were found regularly on seed depots, no observations were made of beetles carrying or destroying seeds.

When certain predator taxa are excluded, other taxa may increase their seed consumption, which may result in >100% contribution to predation due to our method of estimating the contribution of a particular taxon by calculating differences between treatments. For example, the number of seeds consumed of *A. europaeum* in the rodent exclusion treatment exceeded the total number of seeds consumed in the controls, resulting in a contribution of gastropods to seed removal and elaiosome damage of more than 100%. More seeds of *A. nemorosa* were removed from the gastropod and rodent exclusion treatments than from treatments that excluded gastropods only. In this case, cages may have facilitated access to seed depots for insects.

The fate of seeds when fed upon by slugs

In our slug seed feeding experiment, all *Arion* slug species tested readily consumed seeds of the myrmecochores offered. In contrast, *L. cinereo-niger* showed only a low

interest for the seeds. The seeds were not destroyed as only the elaiosomes were eaten or, when seeds were swallowed intact, they were defecated apparently undamaged; *A. europaeum* seeds were defecated with most of the elaiosome still remaining on the seed.

We offered no additional food in our gastropod feeding experiments to avoid satiation of gastropods, with the exception of wet paper towels on which mainly Arionid slugs fed on frequently (Frömmig 1950). In previous studies, unpalatable food was not fed on by slugs after starvation even when no additional food was accessible (e.g. Kozlowska and Kozlowski 2004). We observed the slugs feeding on seeds in the forests where many alternative food sources are available. Thus, we believe that seeds of ant-dispersed plants do belong to the diet of gastropods, mainly Arionid slugs, and that the feeding observed in our laboratory experiments is not due to enforcement.

More seeds of *A. europaeum* than of *A. nemorosa* were consumed by slugs in the laboratory. Ants were found to prefer larger seeds of myrmecochores over smaller seeds (Gorb and Gorb 2003). The seeds of both these plant species, however, are almost of the same size. Ants are attracted by the chemical constitution of the elaiosomes (Gorb and Gorb 2003; Boulay et al. 2006, 2007). Seeds of *A. europaeum* are very attractive to ants (Gorb and Gorb 2003) and, interestingly, they were also very attractive to *Arion* species.

Differences in seed consumption among slug species are likely to be related to differential food preferences of the species. While most *Arion* species have a broad diet spectrum that includes live plants, decaying plant material, fungi, vertebrate carrion, animal excrements and dead invertebrates, *L. cinereo-niger* is considered to feed mainly upon decaying plant material, algae and fungi (Frömmig 1950; Falkner 1989; Bogon 1990). Thus, *Arion* species appear to be more opportunistic in their food choice and are able to exploit a temporary food source, such as seeds. It has also been shown that the fatty acid content of elaiosomes resembles that of the insect haemolymph (Hughes et al. 1994; Fischer et al. 2008) and that mainly predatorous ants collect and consume elaiosomes (Hughes et al. 1994; Servigne and Detrain 2008). It therefore appears reasonable that omnivorous *Arion* species, which also feed on invertebrates, consume the elaiosomes of seeds rather than *L. cinereo-niger*, which is mainly restricted to decaying plant material.

Slugs have been shown to prey on seeds of wildflowers (Hurst and Doberski 2003) and were identified, together with rodents, to be the major seed predators of seeds of Gold-of-Pleasure (*Camelina sativa*) in wildflower strips in northern Switzerland (Kollmann and Bassin 2001). Simonetti et al. (2003) reported that the large slug *Phyllocaulis gayi* was responsible for 14.2% of the predation of *Arachis hypogea* seeds offered in Chilean temperate forests. The slug was also observed to feed on seeds of native trees in the laboratory. The authors concluded that these slugs are a neglected granivore at the study site. Cardina et al. (1996) found the slugs *Arion subfuscus* and *Deroceras reticulatum* to be predators of imbibed Velvetleaf (*Abutilon theophrasti*) and wheat seeds. In contrast to our results, however, these slugs damaged the seed coats of swallowed seeds. For Western Trillium (*Trillium ovatum)* in the North-West of the USA, both slugs and snails were observed to feed exclusively on the elaiosomes of seeds, whereas seeds without elaiosomes were not consumed (Mesler and Lu 1983). Muir (1997) found that *Arion fasciatus* was an important predator of elaiosomes of seeds of the myrmecochorous plant *Asarum canadense* (wild ginger). The slugs neither swallowed seeds nor destroyed seed coats. The findings of these earlier studies are consistent with our finding that slugs feed on elaiosomes.

Seed viability after slug feeding

The large slug *A. rufus* swallowed entire seeds of the two myrmecochores and defecated them apparently undamaged and germinable, suggesting that *A. rufus* may be a potential seed-disperser. Several studies have reported on gastropodochory. Müller (1934) described the dispersal of seeds of a variety of herbs with fleshy fruits by the slug *Arion empiricorum* (synonym *A. rufus*) and that the seeds still germinated after gut passage. Gervais et al. (1998) observed banana slugs (*Ariolimax columbianus*) feeding on wild fruits and swallowing seeds therein. These seeds also germinated after passing through the guts of the slugs. In another study, seeds of Gold-of-Pleasure (*Camelina sativa*) swallowed by slugs germinated from slug faeces (Kollmann and Bassin 2001). Müller-Schneider (1967) observed *A. empiricorum* and different snail species ingesting seeds of the plant Moschatel (*Adoxa moschatellina*) and suggested that this plant is adapted to seed dispersal by gastropods.

In our germination experiment, slug-defecated seeds and seeds with feeding traces germinated at the same rate as control seeds, indicating that seeds do not suffer from consumption by slugs. Müller (1934) compared the germination rates of slug- and snail-defecated seeds and control seeds of some herbs with fleshy fruits. He found that almost equal numbers of seeds germinated under both treatments, but he also found that the germination of Bilberry (*Vaccinium myrtillus)* seeds was accelerated when these had passed through the gut of slugs. The proportion of seeds that germinated between the first and the second check in our germination experiment was comparable among all treatments. We made only two checks, however, and therefore could not definitely confirm that slug

consumption has no influence on the phenology of seed germination in *A. nemorosa*. It has also been shown that for some myrmecochorous plant species, the manipulation of the seed as ants remove the elaiosome (scarification) can increase (e.g. Lobstein and Rockwood 1993) or decrease germination success (e.g., Imbert 2006). This may be the case for slug seed consumption as well.

The potential of seed dispersal by slugs

Whether the elaiosome of seeds of myrmecochores is eaten or the seed is swallowed entirely and therefore may be dispersed appears to depend on a number of factors, including slug size. Individuals of the large *Arion* species swallowed seeds, in contrast to the medium-sized slugs (<80 mm), most of which were juvenile *A. rufus*. However, *L. cinereo-niger* is equal in size to *A. rufus* but only a single individual of the former species swallowed only a single seed of *A. nemorosa*; consequently, there are also species-specific differences unrelated to gastropod size.

Our results suggest that the larger *Arion* species may act as seed dispersers of myrmecochores, while the smaller slugs may act mainly as predators of elaiosomes although the latter may also swallow and disperse smaller seeds of other myrmecochorous plants. It is also possible that seeds could be dispersed over short distances by small gastropod species through the adherence of seeds to their mucus, as observed by Mesler and Lu (1983) for banana slugs (*Ariolimax columbianus*) and seeds of *Trillium ovatum*.

We found seeds of *A. nemorosa*, *A. ranuncoloides and Carex* spec. in the faeces of *A. rufus* individuals caught in the forest at times when the seeds were ripe, demonstrating that slugs naturally swallow and disperse seeds. When gastropods were excluded from seed depots in our field experiment, seed removal was reduced radically. Thus, slugs could be important dispersal agents for myrmecochores primarily in sites where ants are rare or absent.

Gervais et al. (1998) stated that dispersal of seeds by the banana slug may only be a few metres and therefore insignificant relative to the more common dispersal by vertebrates. In the case of slugs dispersing seeds adapted to dispersal by ants, the differences in distance may not be so great. Gomez and Espadaler (1998) reviewed studies on myrmecochorous seed dispersal, and the mean dispersal distance by ants in the northern hemisphere was 0.87 m ($n = 1914$). In our slug dispersal experiment, individuals of *A. rufus* travelled an average linear distance of 4.42 m in a period of 14–18 h, while the median defaecation time of swallowed seeds of *A. europaeum* was 15 h. This is four-fold the distance that seeds are carried by ants, indicating that slug movements are frequent enough to be associated with significant dispersal. However, it has to be considered that slugs might have altered their normal movement behaviour in our experimental conditions.

It has been shown that even small dispersal distances mediated by ants can promote a decrease in seedling aggregation (Kjellsson 1985b; Zhou et al. 2007). Higashi et al. (1989) showed that the mean dispersal distance of 64 cm was enough to reduce seedling mortality in the myrmecochore *Trillium tschonskii* in a temperate broad-leaved deciduous woodland in Japan. In their study on *Asarum europaeum*, Gorb and Gorb (2003) found that even a minimal distance from the parent clone could decrease seedling mortality drastically. Furthermore, slugs move in different directions and defecate the seeds over a period of time, which probably results in a scattered distribution of the seeds; this is in sharp contrast to the clumping of seeds that is often common when dispersal is by ants (Gorb and Gorb 2003). Slugs act as nonstandard dispersers of myrmecochorous seeds. Higgins et al. (2003) demonstrated that long-distance dispersal in plants is usually mediated by nonstandard means of dispersal. Further studies are needed to investigate the role of seed dispersal by slugs in more detail.

One of the most prominent hypotheses in the discussions on the evolution of myrmecochory is the predator avoidance hypothesis (Giladi 2006) in which seeds escape from predation as they are buried by ants inside their nests. Interestingly, control seeds of *A. europaeum* were consumed faster by rodents than seeds from slug faeces when they were offered simultaneously. The difference in time was not great and it has to be tested if such a result can also be obtained in field studies. However, our result indicates that seed dispersal by slugs and subsequent defaecation may result in a reduction of seed predation risk. Boyd (2001) found for *Fremontodendron decumbens* that the main target of rodents was the seed and not the elaiosome. Removal of elaiosomes, however, may also increase seed survival rate as it reduces the ability of rodent predators to locate the seeds (Heithaus 1981; Bond and Breytenbach 1985; Boyd 2001). In our experiments, rodents consumed seeds without elaiosomes within the same time frame as they consumed intact seeds. However, all seeds were offered together on a plate and there was no difficulty for rodents to locate the seeds. More data on seed predation risk of seeds with slug-consumed elaiosomes are needed.

Conclusions

The results of our laboratory experiments and our field observations of slugs feeding on seeds of myrmecochores suggest that slugs could be a major threat to elaiosomes on seeds of ant-dispersed plants and of great importance in terms of seed removal. Thus, more attention should be paid

to the role of gastropods and especially to Arionid or other mainly carnivorous slugs in the seed ecology of myrmecochorous forest herbs. We also found strong indications that slugs can act as seed dispersers of myrmecochores, especially where ants are not very abundant, as is the case of cool and moist beech forests where, however, a diverse community of myrmecochores can be found. Seed consumption and seed dispersal by slugs could reduce rodent seed predation by reducing the attractiveness of seeds to rodents and possibly by reducing the risk that seeds are located by rodents. While we focussed on seeds of myrmecochorous plants, it may well be the case that seeds of non-myrmecochorous plants are also dispersed by slugs. Our work should also provide ideas for further studies on the importance and the contribution of gastropods to the seed ecology of plants in habitats other than beech forests.

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Electronic supplementary material to Manfred Türke et al: Seed consumption and dispersal of ant-dispersed plants by slugs.

Table S1: Plot numbers of experiments in the biodiversity exploratories

Table S1: Experimental plots and grid plots of the biodiversity exploratories project in which the experiments were conducted. The star (*) indicated that the experiment was conducted within 200 m of the surrounding of the plot. A detailed description of the forest plots is available upon request.

Experiment	Year	Plot numbers						
Seed predation in the field	2009	HEW10	HEW11	HEW12	HEW36	HEW42		
Slug dispersal experiment	2007	H12085	H77*	H77*				
Natural seed dispersal by slugs	2008	HEW38*	HEW37*	HEW34*				
	2009	HEW5	HEW6	HEW8	HEW9	HEW10	HEW11	HEW21
Germination experiment	2008	HEW12	HEW40	HEW42				
Preliminary survey: seed predation experiment and slug abundance survey	2007	H20	H22	H24	H29	H65	H68	
		H73	H75	H77	H80	H1251	H1361	
		H1364	H12291	H12907	H12909	H13317	H13319	
		H13321						
Preliminary survey: rodent abundance survey	2007	H1192	H1361	H1420	H12087	H12093	H12297	
		H12503	H12504	H12709	H12913	H13119	H13323	
		H13529	H81136					

Appendix S2: Preferences of *Myrmica rubra* L. ants for seeds of *Anemone nemorosa* and *Asarum europaeum*

Preferences of *Myrmica rubra* L. ants for seeds of *Anemone nemorosa* and *Asarum europaeum* were tested in field cafeteria experiments between 18-08-2009 and 24-08-2009. The experiment was performed on dry grassland on limestone close to the University of Jena (coordinates 11°31’ E/ 50°56’ N). The grassland was bordered by mixed forest stands. In the periphery close to the forest edge the habitat was less dry and ant nests of *Myrmica* spec. were common. In the centre, where the habitat was drier, ant nests of *Formica* spec. became more frequent. We chose *M. rubra* for our experiment as Sernander (1906) mentioned that *M. laevinodis* Nylander (synonym *M. rubra*) was the only ant species he observed to collect the seeds of *A. nemorosa* under natural conditions while seeds of *A. europaeum* are attractive to different ant species (Gorb and Gorb 2003). We offered twenty seeds of each plant species on a 10x10 cm wooden plate (seed depot) simultaneously in 10 cm distance to nests of *M. rubra* (N=10). The seed depot was separated into four 5x5 cm squares. Seeds were arranged in groups of ten of one plant species on two diagonal squares. We recorded the time when each seed was removed from a seed depot by ants. Seed depots were observed for 120 min.

Seeds of *A. europaeum* were removed in all replicates, while seeds of *A. nemorosa* were removed in six replicates only. Significantly more seeds of *A. europaeum* (16±2 mean±SE) were removed from seed depots than of *A. nemorosa* (4±2) within the 120 min observations (Wilcoxon signed ranks test, N=10, Z=2.83, p=0.0048). Seeds of *A. europaeum* were removed on average after 29±2 min (N=149 seeds) and of *A. nemorosa* after 59±5 min (N=41). Twelve seeds of *A. europaeum* were collected by *Formica* spec. which intruded into the area. In two replicates the first seeds of *A. nemorosa* were collected only after all seeds of *A. europaeum* were removed. When unattractive seeds are offered together with attractive seeds this can increase the dispersal rate of the unattractive seeds (Gorb and Gorb 2003).

Gorb E, Gorb S (2003) Seed dispersal by ants in a deciduous forest ecosystem. Kluwer Academic Publishers, Dordrecht

Sernander R (1906) Entwurf einer Monographie der europäischen Myrmekochoren. Kungl Sv Vet-Akad Handl, Stockholm

Appendix S3: Preliminary seed predation experiment and slug abundance survey

The preliminary seed predation experiment was conducted on four days in July (10 to 13-7-2007) and on four days in August 2007 (15 to 16-08-2007 and 22 to 23-08-2007) in the Hainich region (10°27’ E/ 51°05’ N). Nine sites in the unmanaged forest and ten sites in stands of 20-40 year old trees in the age-class forests were chosen for the experiment, with a minimum distance of 140 m between two sites. At each site four seed depots were installed between 8.00 am and 1.00 pm with a minimum distance of 20 m between depots (in total 19x4=58 seed depots). Each depot was supplied with four seeds of *Asarum europaeum*. Seeds were placed on 5x2 cm rubber pads placed themselves in the centre of a 10x10 cm wooden plate exposed on the forest soil. Seed depots were checked the first time at dusk on the same day (8 to 12 hours after installation) and the second time in the morning of the following day (about 24 hours after installation). Slugs that were encountered in the vicinity (within 20 cm) of seed depots at the inspections included two individuals of *Arion rufus*, two individuals of *Limax cinereo-niger* and two individuals of unidentified smaller *Arion* species. The main study species *A. rufus* occurs in the brown and black color morph in the sampling area.

Mucous trails of gastropods were found on plates and rubber pads of eleven seed depots. Traces of rodents (faeces, gnawing traces on rubber pads and dislocation of rubber pads) were found at 40 seed depots. Individuals of *A. rufus* and of smaller unidentified slug species

were observed feeding on *A. europaeum* seeds with individuals of *A. rufus* also swallowing entire seeds. Total seed predation in all 19 plots (removed seeds and seeds without elaiosomes) was 35±5 % (mean±SE) after 12 hours and 89±4 % after 24 hours.

Additionally, right after installation of all four seed depots at one site, all individuals of the slug *A. rufus* that were found on the ground within the 20x20 m plot were counted. The plot was searched using eight transect lines from east to west and west to east. Highly structured areas within the plot were searched more intensively. No dead wood or leaf litter were turned over to search for hidden slugs. The average density of *A. rufus* in twelfe plots sampled in July was 173±34 individuals/ha (mean±SE).

Appendix S4: Preliminary rodent abundance survey

A live-trapping program was performed from 10-09-2007 to 14-09-2007. Five multiple live capture traps (Ugglan type) each were placed at 14 different sites in unmanaged beech forest (Hainich National Park; 10°27’ E/ 51°06’ N). For pre-baiting we used mouse pellets and oats, and traps were kept open for 2 days. Afterwards traps were closed for three successive days. We trapped in the night, and inspected the traps in the morning 12 hours after closing. All animals captured were marked with fur cuts to prevent double counting.

During the three trapping nights we trapped 23 *Myodes glareolus* Schreber (bank vole) and one *Apodemus flavicollis* Melchior (yellow necked mouse). These two species were the only rodent species captured and were chosen for subsequent experiments. Both species are known to feed on seeds of different plant species (Corbet and Ovenden 1980).

Corbet G, Ovenden D (1980) The mammals of Britain and Europe. William Collins Sons & Co Ltd., London

Appendix S5: Effectivity of cages to exclude rodents and slugs

To test if cages used in the field seed predation experiment are suitable to exclude rodents from a food source, we performed an experiment from 24-04-2009 to 03-05-2009 in the laboratory. A live-trapping program was performed from 17-04-2009 to 02-05-2009 in the surrounding of Remderoda field station near the University of Jena (coordinates 11°31’ O/ 50°56’ N) with Ugglan multiple live capture traps. Mouse pellets, grain and apple pieces were used as baits. Traps were checked twice a day. Captured rodents were kept in plastic fauna

boxes (25x40x15 cm), filled with sawdust and closed at the top with a metal grid. Paper towels were offered for nest building. Rodents were fed with mouse pellets and grain. Water was unlimited (drop-bottle). Two hours before the onset of an experiment all food was removed. Two depots (10x10 cm wooden plate) were offered in each fauna box at once, one at each side of the fauna box, with ten barley grains as a food source. Access to one depot was not restricted (controls) while the other depot was covered by a 11x11x4 cm metal cage with a mesh size of 28x10 mm (rhombus-shaped mesh). Depots were placed on a 2 cm layer of flower soil. Cages were dug 0.5 cm deep into the soil to prevent rodents from going under the cage or lifting the cage. Number of grains in each depot was counted every 10 min for 90 min. Another check was made after 720 min. Ten *Apodemus flavicollis* (yellow necked mouse), five were juveniles, and nine *Myodes glareolus* (bank vole), one was juvenile, were tested.

Additionally, we tested from 29-04-09 to 31-04-09 if large slugs can enter the cages. Twenty adult individuals of *Arion rufus* were kept in fauna boxes measuring 27x17x18 cm. One piece of carrot and one piece of potato of standardized size were offered together as the food source at the centre of a 9 cm petri-dish under two conditions, one condition at one side of the fauna box, the other condition on the opposite side. Access to one petri-dish was not restricted, while the other petri-dish was covered by a cage. Fauna boxes were filled with flower soil (2 cm). Cages were dug into the soil. After 24 hours feeding traces on or removal of food were checked.

Rodents were not able to enter cages. More than 95% of grains in controls were consumed within 90 min (*A. flavicollis*: 31±5 min; *M. glareolus*: 41±9 min (mean±SE)). As flower soil was rather loose and cages could not be fixed to the ground adequately, however, rodents were able to lift cages in some cases. Rodents lifted cages in two replicates within 90 min and consumed the grains and in seven additional replicates cages were shifted and grains were consumed on the check after 720 min. In contrast, cages can be fixed to forest soil sufficiently and are unlikely to be lifted by rodents then. We conclude that the cages we used effectively exclude rodents in forest field experiments, even juveniles.

Cages did not reduce access rate of slugs to the food source (N=4 not feeding, N=16 slugs feeding) compared to the control group (N=5 not feeding, N=15 feeding, χ^2=0.14; df=1; p=0.71).

Appendix S6: Effect of slug repellent on slugs

Slug repellents were tested in a laboratory experiment in June and July 2008. Slugs were kept in fauna boxes measuring 27x17x18 cm (Savic Fauna box – 6 L.; http://www.savic.be). One piece of carrot and one piece of potato of standardized size were offered together as the food source at the centre of a 10x10 cm wooden plate. The plate was placed in one half of the fauna box with 2.5 cm distance to each border of the box. For one group of individuals (*A. rufus* N=36 and *L. cinereo-niger* N=16) access to the food source was not restricted. For another group (*A. rufus* N=36 and *L. cinereo-niger* N=16) slug deterring copper wire (SNA.P Snail Protect, Gartenring) was attached to the wooden plate and covered with slug repellent (Schneckenabwehrpaste, IRKA). The copper wire and slug repellent were not in contact with the food. After 24 hours feeding traces on or removal of food were checked.

Occasionally slugs were able to cross the repellent but slug repellent significantly reduced access rates to the food source for *A. rufus* (N=29 not feeding, N=7 slugs feeding) compared to the control group (N=7 not feeding, N=29 feeding, χ^2=24.50; df=1; p<0.001) and for *L. cinereo-niger* (N=15 not feeding, N=1 feeding) compared to the control group (N=6 not feeding, N=10 feeding, χ^2=8.87; df=1; p=0.0029).

Appendix S7: Effect of slug repellent on ants

Slug repellents were tested for their effect on ants in a field experiment on 18-08-2009, starting at 9:35 am. Weather was dry and warm (29°C maximum). The experiment was performed on dry grassland on limestone close to the University of Jena (coordinates 11°31' O/ 50°56' N). The grassland was bordered by mixed forest stands. In the periphery close to the forest edge the habitat was less dry and ant nests of *Myrmica* spec. were common. In the centre, where the habitat was drier, ant nests of *Formica* spec. became more frequent. We offered tuna baits on 10x10 cm wooden plates in two transect lines at six positions in each transect. Transects were separated by 5 m and positions were also separated by 5 m. Transects started close to the forest edge, leading to the centre of the dry grassland. Two depots with tuna baits were offered at each position, while access to one depot was not restricted (controls), in the other depot slug deterring copper wire (SNA.P Snail Protect, Gartenring) was attached to the wooden plate and covered with slug repellent (Schneckenabwehrpaste, IRKA). Depots were checked every ten minutes, ants on seed depots were counted (1-20 ants of the same genus) or categorized as more than 20 ants of the same genus (20 ants were used for calculations of mean ant numbers in depots). After

one hour, control depots were removed while treatment depots remained for another 60 minutes.

Ants were deterred by the slug repellent. While ants were present in every control depot on at least some checks (9.0±0.9 ants per depot; mean±SE for all control depots and all checks in 60 min), no ants were found within depots protected by slug repellent on any check (120 min). Ants of the genera *Myrmica*, *Formica* and *Tapinoma* were attracted to tuna baits. Four control depots were only visited by *Myrmica* spec. All other depots were visited by species of two or three genera. *Tapinoma* spec. was only abundant with many workers in one depot on the 10 min (12 workers) and in another depot on the 20 min checks (17 workers) and afterwards only few individuals visited five depots, which were mostly dominated by *Formica* spec. or *Myrmica* spec. After removal of controls and occasionally before the removal, ants were observed trying to access protected depots but ants always retreated when they came into contact with the slug repellent. Ants were climbing the vegetation above the protected seed depots, trying to reach the tuna bait. We conclude that the slug repellent is suitable to exclude ants from a food source.

Appendix S8: Effectivity of bridges for ants to cross slug repellent

We tested in a field experiment from 04-08-09 to 24-08-09 if seed depots applied with slug repellent and ant bridges allow ants to enter seed depots and to remove seeds as slug repellent also deterred ants (see Appendix S7). Experiments were started in the morning on warm dry days. Ants were active during experiments. The experiment was performed on dry grassland on limestone close to the University of Jena (coordinates 11°31’ O/ 50°56’ N). The grassland was bordered by mixed forest stands. We offered ten seeds of *Asarum europaeum* on 10x10 cm wooden plates (seed depot) in 10 cm distance to the entrance of an ant nest of *Myrmica rubra*. Ten nests were tested. We exposed seed depots under two treatments to each ant nest: control: access to the seed depot was not restricted; slug repellent treatment: slug deterring copper wire (SNA.P Snail Protect, Gartenring) was attached to the wooden plate and covered with slug repellent (Schneckenabwehrpaste, IRKA); in all four directions of the seed depot, a 18 cm long bridge made of zinc coated 2.8 mm strong wire was dug into the ground with one end and the other end was leading to the centre of the seed depot, making it possible for ants to cross the repellent and to enter the seed depot. Treatments were tested one after the other, starting with the slug repellent treatment. Controls were offered on the opposite side of the ant nest in 180° to the slug repellent treatment. Seed depots of the slug repellent treatment were observed for 180 min and controls were observed

for 90 min, unless all seeds were removed earlier. We registered the time when ants entered a seed depot and also the time when each seed was removed from a depot.

Ants entered eight of ten seed depots protected by slug repellent over the bridges (first individual after 31±10 min (mean±SE)). All control seed depots were entered by ants (first individual after 7±2 min). Ants removed a total of eleven seeds after 112±13 min from three slug repellent treatments. Seven seeds were removed by *Formica* spec. In controls, 89% of seeds were removed within 90 min by ants, in average after 29±2 min. Only three seeds were removed by *Formica* spec. Significantly more seeds were removed from controls than from protected treatment depots (Wilcoxon signed ranks test: N=10, Z=2.84, p=0.004). Four ants were observed to cross the repellent directly, but did not carry seeds over the paste. Sometimes ants took several minutes trying to carry seeds over bridges.

Appendix S9: Effect of slug repellent on rodents

To test the effect of slug repellents on the feeding behaviour of rodents we performed an experiment from 25-04-2009 to 03-05-2009 in the laboratory. A live-trapping program was performed from 17-04-2009 to 02-05-2009 in the surrounding of Remderoda field station near the University of Jena (coordinates 11°31’ O/ 50°56’ N) with Ugglan multiple live capture traps. Mouse pellets, grain and apple pieces were used as baits. Traps were checked twice a day. Captured rodents were kept in plastic fauna boxes (25x40x15 cm), filled with sawdust and closed at the top with a metal grid. Paper towels were offered for nest building. Rodents were fed with mouse pellets and grain. Water was unlimited (drop-bottle). Two hours before the onset of an experiment all food was removed. Two depots (10x10 cm wooden plate) were offered in each fauna box at once, one at each side of the fauna box, with ten barley grains as a food source. Access to one depot was not restricted (controls) and in the other depot slug deterring copper wire (SNA.P Snail Protect, Gartenring) was attached to the wooden plate and covered with slug repellent (Schneckenabwehrpaste, IRKA). Number of grains in each depot was counted every 10 min for 110 min. Another check was made after 720 min. Grains that were not consumed after 110 min but after 720 min were given the value 120 min for statistics. Time of consumption of grains (=time of the check on which the grain was consumed) was compared between controls and protected depots by split-plot ANOVA. Nine *Apodemus flavicollis* (yellow necked mouse), four were juveniles, and six *Myodes glareolus* (bank vole) were tested.

All grains were consumed after 720 min and 89% were consumed within the first 110 min (6% of grains in controls and 15% in protected depots were consumed after more than 110 min). Grains in controls were consumed after 40±2.5 min (mean±se) and in protected depots after 68±2.9 min. Grains in control depots were consumed faster than grains protected by slug repellent (Split-plot ANOVA, $F_{1,15}$ =5.79; p<0.05). There was no difference between rodent species in the time to consumption of grains ($F_{1,15}$ =0.34; p>0.05). Rodents were sometimes observed to sit within protected depots while consuming grains, and in some cases rodents left the depot with the grain to consume it. Though grains in control depots were consumed first by most individuals, grains in depots surrounded by slug repellent were not protected against rodent feeding.

Appendix S10: Ant survey

To confirm our observations and the evidence from literature that ants are rare in mature beech forests, such as the Hainich National Park, we compared data from pitfall traps between different forest land-use types and developmental stages. Traps were installed in 50 experimental plots in the Hainich region in the surrounding of Mühlhausen in Thuringia, Germany, at 400 to 500 m a.s.l. (10°27’ O/ 51°05’ N) between March and April 2008. This study is part of the biodiversity exploratories project (http://www.biodiversity-exploratories.de/). More detailed information on the sampling area and research plots can be provided upon request. Plots were set up in unmanaged beech forest (Hainich National Park), age-class beech forest of different developmental stages (see table S11), selection cutting beech forest and age-class spruce forest (young timber). We installed pitfall traps of 15 cm diameter with a funnel, protected by a 25x25 cm plastic roof in three corners of each 100x100m plot. Traps were filled to 60% volume with 3% copper sulphate and soap to decrease the surface tension. Arthropods were trapped from May to October. Traps were emptied monthly. Arthropods in samples were sorted and ants were counted. Samples from each corner sub-plot from May to October were sorted in twelve of 50 plots, the so called VIPs (very intensive research plots; for distribution of VIPs on different land-use types see table S11), and two samples per month from June to October were sorted in the other 38 plots (experimental plots). Due to losses by trap damages, in some cases fewer samples were available and in twelve cases all three samples of a monthly collection were sorted in experimental plots. All specimens of ants were identified to species level from samples in the VIPs. Pitfall traps are commonly used for ant trapping and are a good method for sampling ant communities (Vele 2009). There is no difference in efficiency to sample ants in pitfall traps with or without a funnel (Obrist and Duelli 1996).

The results of the ant survey are summarised in Table S11. In almost half of the plots in the unmanaged beech forest, no ants were sampled. In the other half of the plots, ants were trapped at least in a single sample. Only single individuals or not more than two individuals per sample were collected. Ant workers in the unmanaged forest included *Myrmica ruginodis*, the small ant *Stenamma debile* and the arboreal, mostly trohobiontic ant *Lasius brunneus*. A list of ant species in the different land-use types is shown in Table S12. The unmanaged beech forest had the lowest proportion of samples with ants and the lowest number of ant individuals per sample with ants compared to the other forest land-use types. Ants were more often trapped and more ant species were sampled in young developmental stages of the age-class forest than in older stages.

Obrist MK, Duelli P (1996) Trapping efficiency of funnel- and cup-traps for epigeal arthropods. Mitt Schw Ent Ges 69:361-369

Vele A, Holusa J, Frouz J (2009) Sampling for ants in different-aged spruce forests: A comparison of methods. Eur J Soil Biol 45:301-305

Table S11: Presence of ants in forests in the Hainich region

Table S11: Ants trapped by pit-fall traps in different forest land-use types in the Hainich region.

Forest land-use type Developmental stage	Spruce Young timber	Beech age-class Thicket	Beech age-class Thicket w. shelterwood	Beech age-class Pole wood	Beech age-class Young timber	Beech age-class Old timber	Beech selection cutting Uneven-aged	Beech unmanaged Uneven-aged
No. plots (VIP*)	4 (3)	5 (1)	3	4	4 (1)	4 (1)	13 (3)	13 (3)
plots with ants (%)	100	100	100	100	75	75	77	54
No. samples	65	60	32	40	51	53	156	147
No. samples with ants	28	49	17	12	5	11	38	14
samples with ants/ samples (%)	43	82	53	30	10	21	24	10
mean no. ants/ sample with ants	7.5	13.3	7.8	4.0	3.5	2.6	3.0	1.4

*VIP, very intensive research plot

Table S12: Ant species sampled in forests in the Hainich region

Table S12: Ant species trapped by pit-fall traps in the very intensive research plots (VIPs) in different forest land-use types in the Hainich region.

Forest land-use type Developmental stage	Spruce Young timber	Beech age-class Thicket	Beech age-class Young timber	Beech age-class Old timber	Beech selection cutting Uneven-aged	Beech unmanaged Uneven-aged
No. plots (VIP)	3	1	1	1	3	3
No. samples	54	18	18	18	53	52
Ant species						
Workers	*Formica polyctena* *Lasius brunneus* *Lasius platythorax* *Myrmica ruginodis*	*Lasius niger* *Lasius platythorax* *Myrmica ruginodis*	*Myrmica ruginodis*	no ants	*Myrmica ruginodis*	*Lasius brunneus* *Myrmica ruginodis* *Stenamma debile*
Queens	*Camponotus ligniperda* *Lasius fuliginosus* *Lasius niger* *Lasius platythorax* *Myrmica ruginodis*	*Lasius flavus* *Lasius fuliginosus* *Lasius platythorax* *Lasius umbratus* *Myrmica ruginodis* *Myrmica ruginodis*			*Myrmica ruginodis*	*Lasius niger*
Males	Formicinae Myrmicinae					

CHAPTER THREE

The Seed Dispersal Potential of Some Gastropod Species and Indications for Predator-Avoidance of Slug-Consumed Seeds

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Abstract

Seed dispersal of ant-dispersed plants (myrmecochores) is a well studied ecosystem function. Recently, slugs have been found to act as seed dispersers of two myrmecochores and slug-defecated seeds were less attractive to rodents in the laboratory. The aim of our study was to (1) generalize the finding that gastropods feed on seeds of myrmecochores and hence may act as seed dispersers and to (2) test the hypothesis that slug-defecated seeds increase the chance of seeds to escape predation.

We assessed the seed dispersal potential of four slug and snail species with a set of seven myrmecochorous plant species from different families common to Central European beech forests. Seeds differed in shape and size. Gastropods differed in their readiness to feed on seeds and in the proportion of seeds that were swallowed as a whole, and this readiness was negatively correlated with seed size. Smaller slugs mostly fed on the elaiosome but also swallowed small seeds and therefore not only act as elaiosome predators but may also disperse seeds. Large slugs swallowed the majority of seeds in some plant species that were defecated without damage. Native red slugs (*Arion rufus)* showed a similar feeding behavior as the invasive Spanish slug (*Arion lusitanicus)* which is currently out-competing the native slug. In a seed removal experiment with seeds of wood anemone (*Anemone nemorosa*) in beech forests in Central Germany, 78% of the untreated seeds were removed from seed

depots within eight days, while only 54% of the seeds with feeding traces of slugs and 32% of slug-defecated seeds were removed.
Our results help to generalize the finding that gastropods consume and potentially disperse seeds of myrmecochores and that one benefit for plants might be seen in predator-avoidance of seeds that gastropods had fed upon.

Introduction

Seed dispersal by ants (myrmecochory) was a driver of an incredible diversification of angiosperms (Lengyel et al. 2009). Thousands of plant species worldwide rely on ants as dispersal vectors of their diaspores and many ant-dispersed plant species, called myrmecochores, are coexisting in certain habitats (Beattie 1985; Sernander 1906; Ulbrich 1919). Dispersal of seeds by ants allows plants to reach microsites suitable for seedling establishment (Beattie 1985; Giladi 2006; Gorb and Gorb 2003) and helps seeds to escape fire or predation by the rapid removal or by the burial of seeds (Anderson and MacMahon 2001; Bond and Slingsby 1984; Bond and Breytenbach 1985; Boyd 2001). In a recent study by Türke et al. (2010) slugs have been identified as seed dispersers of forest myrmecochores. While myrmecochory has been studied for more than one hundred and forty years now (see references in Sernander 1906), we cannot tell if the same rules can be applied to seed dispersal by gastropods (gastropodochory).

With about 35,000 species, terrestrial gastropods are a very diverse group, with different foraging behavior and feeding habits (Barker 2001; Bogon 1990). Slugs have been shown to consume the elaiosome, a nutrient-rich appendage on seeds, without dispersing the seed (Mesler and Lu 1983; Türke et al. 2010). However, the red slug, *Arion rufus*, has recently also been shown to swallow the rather large seeds of wood anemone (*Anemone nemorosa* L.) and European wild ginger (*Asarum europaeum* L.) in the laboratory and in the field (Türke et al. 2010). Gastropod-defecated seeds were undamaged and germinable and thus dispersed endozoochorously (Gervais et al. 1998; Honek et al. 2009; Müller(-Schneider) 1934; Türke et al. 2010). Thus, gastropods display different functional traits, acting either as mutualists or antagonists for plants and this may vary between and within gastropod and plant species. The elaiosome has been shown to have some chemical similarity to the insect hemolymph and in ants it has been demonstrated that mainly predatory species forage for myrmecochorous seeds (Fischer et al. 2008; Hughes et al. 1994; Servigne and Detrain 2008). If this also applies to gastropods, one could suggest that predatory gastropods feeding on invertebrates rather than strict herbivores should feed on myrmecochorous seeds. In fact, recent results supported this hypothesis, but the number of gastropod and plant

species involved in the study, however, were too limited to generalize this assumption (Türke et al. 2010). On the other hand, Honek et al. (2009) found that ten slug and snail species with different feeding habits consumed wind-dispersed seeds of Dandelion (*Taraxacum* agg.) in the laboratory, which lack an elaiosome. Thus, we cannot exclude that the seed itself is somehow attractive to gastropods.

Benefits of gastropodochory for the plant could potentially be greater dispersal distances permitted by slugs than by ants or the availability of slugs as seed dispersers where ants are rare (Türke et al. 2010). In addition, dispersal by slugs may reduce the subsequent risk of seed predation as seeds swallowed and defecated by slugs were less attractive to rodents than untreated seeds in the laboratory (Türke et al. 2010).

As a first step in solving the complexity of this previously unknown mutualism and to test the generality of gastropodochory in myrmecochores, we conducted feeding experiments in the laboratory with seeds of seven forest myrmecochores, differing in size and shape, offered to four gastropod species with different feeding habits.

We conducted a removal experiment with untreated seeds, slug-defecated seeds and seeds with feeding traces by slugs in managed and unmanaged beech forests to test whether seeds fed upon by slugs are less likely to be removed by animals from seed depots.

Materials and methods

Plant material

Seeds of seven myrmecochorous spring geophytes (Gorb and Gorb 2003; Sernander 1906; Servigne 2008; Ulbrich 1919) were collected in deciduous forests in the Hainich region south of Mühlhausen in Thuringia, Germany, at 400 to 500 m a.s.l. (coordinates 10°27’ O/ 51°05’ N) and in deciduous forests surrounding Jena, Thuringia (11°36’ O/ 50°56’ N). Seeds were collected just before ripening (April to June) and kept in a freezer at -20° C until used in the experiments. Seeds of (1) bear’s garlic (*Allium ursinum* L.; Alliaceae) lack a distinct elaiosome but seeds are surrounded by a fatty seed coat. Seeds of (2) toothwort (*Lathraea squamaria* L.; Scrophulariaceae), (3) hedge violet (*Viola reichenbachiana* Boreau; Violaceae), (4) wood anemone (*Anemone nemorosa* L.; Ranunculaceae), (5) dog’s mercury (*Mercurialis perennis* L.; Euphorbiaceae), (6) European wild ginger (*Asarum europaeum* L.; Aristolochiaceae) and (7) yellow archangel (*Lamiastrum galeobdolon* agg. L.; Lamiaceae) all bear a more or less distinct elaiosome.

Seeds were measured digitally at 12.5 times magnification with the program COMEF_Autoshape 3.0 (OEG GmbH) using a MZM1 microscope (Mikroskop Technik

Rathenow), a CF11DSP camera (Kappa optronics GmbH) and a FALCON Framegrabber (IDS Imaging Development Systems GmbH). Maximum width (W), length (L) and thickness (T) of the diaspores (seed including elaiosome) and of the elaiosome of ten randomly chosen seeds of each species were used to calculate seed volume (V) as W × L × T. For *A. nemorosa,* the diaspore volume was calculated as W × L × T of the achene's body + W × L × T of the style. The elaiosome is rather indistinct in *A. nemorosa* and in *A. ursinum*. We did not measure the elaiosome in these two species. Notes on the seed shape were partly derived from Bojňanský and Fargašová (2007). Seeds of species from seven different plant families with different sizes and shapes were chosen to test the generality of our results.

Slugs and snails

Experiments were conducted with native gastropods abundant in beech forests, in particular, mature and juvenile red slugs *(Arion rufus* L.), ash-grey slugs *(Limax cinereo-niger* Wolf) and white-lipped snails (*Cepaea hortensis* Müller) and additionally with the invasive Spanish slug (*Arion lusitanicus* Mabille). All gastropods were collected in deciduous forests in the Hainich region except for *A. lusitanicus* which was collected in a garden in Hermsdorf, Thuringia, about 40 km east of Jena. All mature slugs were larger than 10 cm when stretched. Juvenile *A. rufus* measured 58±4 mm (mean±SE). Species were chosen due to their abundance in beech forests (Müller et al. 2005) and their size as we supposed that all species tested could potentially disperse seeds.

Gastropods were kept in the climate chamber at 20°C and 75% humidity. Slugs of more than 5 cm body length were kept in fauna boxes measuring 27 × 17 × 18 cm L × W × H (Savic Fauna box – 6 L.; http://www.savic.be). Slugs of less than 5 cm body length and snails were kept in 9 cm petri-dishes. Gastropods were fed once a week with lettuce, carrots and wild herbs. All food was removed one day before an experiment. Gastropods were kept on wet paper towels, which most gastropods fed on frequently.

Gastropod seed preferences

Gastropod individuals were offered six seeds of one plant species at a time except for *A. europaeum* where only five seeds were offered to *A. rufus*. Seeds of *L. galeobdolon* were only offered to mature and juvenile *A. rufus* as numbers were limited. On average, seeds of a plant species were offered to 14 ± 1 individuals (mean±SE) of a species (Table 1). In total we used three gastropod species × 6 plant species = 18 plus additionally two age classes of *A. rufus* × 7 plant species = 14, in total 32 gastropod-plant combinations. Experiments were conducted from August to October, 2007.

Seeds were exposed to gastropods for 48 hours and were checked after 24 and 48 hours and the outcome was classified as follows: (1) swallowing of the whole seed, (2)

consumption of elaiosome only, (3) no feeding (unregarded). The numbers of seeds offered of a plant species that were swallowed or of which only elaiosomes were consumed per individual was used for statistical analyses. Some individuals were offered seeds of several plant species and we accounted for the re-use of individuals in the analysis. In case seeds were apparently undamaged, they were examined under the microscope at 65-times magnification. Feces were collected after 24 hours and 48 hours to search for digested and defecated seeds. Defecated seeds were also examined under the microscope.

There were differences in the way seeds of different plant species were consumed: in *A. ursinum, L. squamaria* and *V. reichenbachiana* no feeding traces on the seed coat or on elaiosomes were visible although feeding cannot be entirely excluded as slugs and snails appeared to handle seeds. For these plant species, therefore, only the proportions of seeds swallowed were analysed. For seeds of *A. nemorosa, M. perennis, A. europaeum* and *L. galeobdolon* feeding on the elaiosomes was observed and both, elaiosome feeding and swallowing of entire seeds, were used in the analysis.

Results of the feeding experiments with *A. nemorosa* and *A. europaeum* by mature and juvenile *A. rufus* and by *L. cinereo-niger* were published in Türke et al. (2010) (Türke et al. 2010). These original data were integrated in our multi-species analysis.

Table 1. Number of gastropod individuals to which seeds of certain plant species were offered (No) and number of individuals that fed on the seeds (Nf).

	A. rufus (mature)		*A. rufus* (juvenile)		*L. cinereo-niger*		*A. lusitanicus*		*C. hortensis*	
	No	Nf	No	Nf	No	Nf	No	Nf	No	Nf
Elaisome damage not visible										
A. ursinum	16	6	5	0	17	0	13	5	11	0
L. squamaria	16	10	10	5	17	0	8	6	12	1
V. reichenbachiana	15	10	9	1	19	3	11	10	12	0
Elaisome damage visible										
A. nemorosa	21	12	13	7	19	3	15	9	13	7
M. perennis	21	19	10	9	18	16	9	9	13	11
A. europaeum	28	28	13	9	19	3	17	8	13	5
L. galeobdolon	19	9	9	8	-	-	-	-	-	-

In species where elaiosome damage was visible, feeding included swallowed seeds and seeds with consumed elaiosomes and in species where elaiosome damage was not visible, only swallowed seeds are regarded as fed upon.

Removal of slug-consumed seeds in the field

A seed removal experiment was conducted on June 23rd 2009 in 10 experimental plots in beech forests in the Hainich region within the framework of the biodiversity exploratories project (Fischer et al. 2010). We included five plots in an unmanaged forest, the Hainich National Park, and five plots in age-class forests (three in old and two in young timber) where all the trees are of the same age. Seeds (achenes) of *A. nemorosa* were offered in groups of ten on a 10 × 10 cm wooden plate (seed depot), protected against rain by a 12.5 × 12.5 cm

plastic roof but not against animals. Seeds of three different treatments were offered: (A) untreated control seeds; (B) defecated seeds: seeds which had previously been swallowed and defecated by red slugs (individuals of *A. rufus* were collected in forests in the Hainich and seeds were fed to them in the laboratory in June 2009); (C) seeds with feeding traces: seeds of which slugs had consumed parts of or the whole fruit skin of the achene (with 15 to 100 % of the fruit skin area consumed). Depots were arranged in a triangle and separated by four meters. One replicate of each set of treatments was offered in each of two different corners of a 100 × 100 m plot. Seeds were counted after 1, 2, 4 and 8 days.

Statistical analysis

Statistics were performed with R 2.8.0 (R Development Core Team; http://www.r-project.org/). Diaspore and elaiosome volumes as well as the elaiosome-diaspore-volume-ratio of plant species were transformed to obtain normality and compared with ANOVA using the function “aov”. In the gastropod feeding experiment, we compared the number of swallowed seeds (bound to the number of seeds not swallowed by the function “cbind”) and of seeds with consumed elaiosomes (bound to the sum of seeds not consumed and of seeds swallowed) between gastropod species and plant species in a generalized linear mixed-effects model (fit by the Laplace approximation for binomial errors) using the function “lmer” in the lme4 package. Plant species and gastropod species were treated as fixed effects and gastropod individuals as a random effect. The number of swallowed seeds was further analyzed in a GLMM for its dependency on diaspore volume (fixed effect) with gastropod individual as a random effect.

In the field seed removal experiment, we compared the number of removed seeds (bound to the number of seeds not removed by the function “cbind”) between different treatments with data from all checks (1,2,4 and 8 days) in a GLMM (binomial errors) using the function ‘‘lmer’’. Treatment was a fixed effect and subplots (plot corners) were nested in plots and treated as the error and the date of each check as an additional error. We performed multiple comparisons of means (Tukey contrasts) for all tests using the function ‘‘glht’’ in the multcomp package.

To obtain a simplified ranking of the “dispersal” frequency or of the elaiosome damaging frequency of the gastropod species, we calculated for each gastropod species a) the proportion of seeds swallowed and b) the proportion of seeds where only the elaiosome was consumed. Proportions were first averaged over all trials of each plant species and then averaged over plant species. We used a similar procedure to rank plant species according to the frequency at which seeds were “dispersed” by gastropods and to the elaisome damaging frequency of gastropods with the mean of the proportions of each gastropod species.

Field work permits

Field work permits were given by the responsible state environmental offices of Thüringen (2007: Hainich National Park and Landratsamt Unstrut-Hainich-Kreis, Bau und Umwelt, Untere Naturschutzbehörde, Aktenzeichen (AZ) 13.464233/11-07SDH; 2009: Hainich National Park and Landratsamt Unstrut-Hainich-Kreis, Bau und Umwelt, Untere Naturschutzbehörde, AZ 2620/AUSN-02-09 and Landratsamt Eichsfeld, 70.2 – Natur- und Artenschutz, AZ 70.2-4-700/G1-002/09).

Results

Seed dimensions

Plant species differed significantly in diaspore volume (V_D; seed including elaiosome; ANOVA; $F_{6,63}$=60.08, p<0.001), except for the pairs of *A. ursinum* and *L. galeobdolon* and also *A. ursinum* and *A. europaeum* (Table 2). Seeds included very small (*L. squamaria*), small (*V. reichenbachiana*) and medium-sized to large seeds (*A. ursinum, A. nemorosa, A. europaeum, L. galeobdolon, M. perennis*) and seeds differed in shape, too. Plant species, excluding species with indistinct elaiosomes (*A. nemorosa* and *A. ursinum*), differed significantly in elaiosome volume (V_E; ANOVA; $F_{4,45}$= 79.34, p<0.001; Table 2) and in the ratio of the elaiosome and the diaspore volume (V_E/V_D; ANOVA $F_{4,45}$= 28.24, p<0.001; Table 2).

Table 2. Mean diaspore (seed including elaiosome) and elaiosome dimensions (in mm; mean of ten seeds per species) and diaspore shape.

	Diaspore				Elaiosome					
Plant species	L	W	T	V_D	L	W	T	V_E	V_E/V_D	Shape
L. squamaria	1.3	1.2	1.1	1.8	0.3	0.6	0.3	0.1	0.06	± spherical
V. reichenbachiana	2.4	1.6	1.5	5.8	0.6	0.6	0.5	0.2	0.03	obovoid
A. nemorosa	4.9	2.1	1.6	16.5	-	-	-	-		ellipsoid, flattish, hairy
L. galeobdolon	4.5	2.2	1.7	17.2	2.0	0.8	1.2	2.0	0.12	trigonous, obovate, dorsal convex
A. ursinum	3.1	3.0	2.3	21.4	-	-	-	-		± spherical, truncate below
A. europaeum	4.2	2.5	2.1	22.1	4.0	1.3	1.1	5.6	0.25	obovate, dorsal convex, ventral concave
M. perennis	3.4	3.0	3.0	31.8	3.0	0.6	1.2	3.1	0.10	sperical
		(mm)		(mm^3)		(mm)		(mm^3)		

L=maximum length; W=maximum width; T=maximum thickness; V=volume as LxWxT; V_D= diaspore volume; V_E=elaiosome volume; obovate=egg shaped in outline; obovoid=hen's egg shape

Gastropod seed preferences

Individuals of all gastropod species fed on seeds of at least some plant species (Fig. 1). Only a part of the gastropods to which seeds of certain plant species were offered fed on the seeds (Table 1). Linear mixed-effects models revealed significant differences in numbers of swallowed seeds (N=487 feeding trials involving N=156 gastropod individuals) and in numbers of seeds with consumed elaiosomes (N=287 feeding trials involving N=138 gastropod individuals) between plant species and between gastropod species (for details of the GLMM (Tukey contrasts) see Table 3). Diaspore size was negatively correlated with the proportion of seeds swallowed (GLMM; $z=-8.18$, $p<0.001$; Fig. 2). *Asarum europaeum*, however, was an exception. Its seeds are among the largest in the set but the large Arionid slugs swallowed a much higher proportion of them than of the other seeds offered.

Swallowed seeds that were defecated by snails or slugs were all intact. Except for *M. perennis* and *A. nemorosa,* elaiosomes were also in most cases apparently undamaged after gut passage. In *A. nemorosa* seeds, the fruit skin not only of the basal part of the peduncle (which should denote the elaiosome (Canullo 1985)) was consumed but also of other parts (including the style). We considered all of this feeding on *A. nemorosa* as elaiosome damage.

Seeds of *Mercurialis perennis* suffered mostly elaiosome predation while only few of these large seeds were swallowed by slugs (Fig. 1, Fig. 3). The swallowing of seeds of *A. europaeum* by the large Arionid slugs was disproportionately high.

We ranked all gastropod species and all plant species according to the seed swallowing frequency and to the elaiosome damaging frequency in Figure 3: among the gastropods, the large *Arion* individuals are probably most important for seed dispersal. Juvenile *A. rufus* only swallowed small seeds and feeding on large seeds was restricted to the consumption of the elaiosomes, suggesting difficulties of swallowing large seeds. *Limax cinereo-niger* and *C. hortensis* were less frequently feeding on seeds, mostly consumed elaiosomes and swallowed only very few small seeds.

Removal of seeds fed on by slugs in the field

A total of 326 of the 600 seeds exposed (54%) were removed from seed depots within eight days. More control seeds (156 out of 200, 76%) were removed than seeds with feeding traces (107 out of 200, 54%, GLMM with Tukey post-hoc test; $z=11.59$, $p<0.001$, Fig. 4) and defecated seeds (63 seeds, 32%, $z=17.49$, $p<0.001$), and more seeds with feeding traces were removed than defecated seeds ($z=9.89$, $p<0.001$). 36 of the 44 remaining control seeds had in fact feeding traces and four seeds were moldy. There was a tendency that more seeds were removed in the unmanaged forest than in the age-class forests, independent of seed treatment, but the differences were not significant ($z=1.87$, $p=0.061$).

Within one day there were mucous trails of slugs and snails on 82% of the seed depots, suggesting that most seed depots were visited by gastropods. On only 12% of the seed depots, no gastropod traces were found at the end of the experiment. Despite the short control intervals, a total of 30 gastropods were encountered on seed depots: 21 *A. rufus*, seven *Arion* spec. of less than 5 cm body length and two snails.

Table 3. Pairwise comparisons of gastropod and plant species in the way seeds are fed on by gastropods. Results of GLMM analysis (Tukey contrasts) for differences in seed consumption of seven plant species by four gastropod species in the gastropod seed preference experiment. Asterisks indicate significant differences between pairs: *, p<0.05; **, p<0.01; ***, p<0.001.

		No. of swallowed seeds		No. of seeds with elaiosome damage	
Comparison of plant species		z	Pr(>|z|)	z	Pr(>|z|)
A. nemorosa	*M. perennis*	4.42	< 0.001 ***	-13.47	< 0.001 ***
	A. europaeum	-5.01	< 0.001 ***	0.03	1.00
	L. galeobdolon	0.92	0.97	1.56	0.39
	A. ursinum	4.45	< 0.001 ***		
	L. squamaria	-4.32	< 0.001 ***		
	V. reichenbachiana	-2.43	0.18		
M. perennis	*A. europaeum*	-7.78	< 0.001 ***	13.18	< 0.001 ***
	L. galeobdolon	-2.91	0.052 .	8.29	< 0.001 ***
	A. ursinum	-0.36	0.99		
	L. squamaria	-7.57	< 0.001 ***		
	V. reichenbachiana	-6.06	< 0.001 ***		
A. europaeum	*L. galeobdolon*	4.19	< 0.001 ***	-1.54	0.41
	A. ursinum	8.15	< 0.001 ***		
	L. squamaria	0.14	1.00		
	V. reichenbachiana	2.41	0.19		
L. galeobdolon	*A. ursinum*	2.64	0.11		
	L. squamaria	-3.99	0.0012 **		
	V. reichenbachiana	-2.63	0.11		
A. ursinum	*L. squamaria*	-7.74	< 0.001 ***		
	V. reichenbachiana	-6.37	< 0.001 ***		
L. squamaria	*V. reichenbachiana*	2.11	0.33		
Comparison of gastropod species					
A. rufus (mature)	*A. rufus* (juvenile)	4.83	< 0.001 ***	-3.63	0.0026 **
	L. cinereo-niger	6.83	< 0.001 ***	1.08	0.81
	A. lusitanicus	-1.40	0.59	-2.01	0.26
	C. hortensis	4.17	< 0.001 ***	1.69	0.43
A. rufus (juvenile)	*L. cinereo-niger*	2.46	0.09 .	3.96	< 0.001 ***
	A. lusitanicus	-5.06	< 0.001 ***	1.42	0.61
	C. hortensis	2.19	0.16	4.21	< 0.001 ***
L. cinereo-niger	*A. lusitanicus*	-6.95	< 0.001 ***	2.60	0.069 .
	C. hortensis	0.79	0.92	0.68	0.96
A. lusitanicus	*C. hortensis*	4.48	< 0.001 ***	2.99	0.023 *

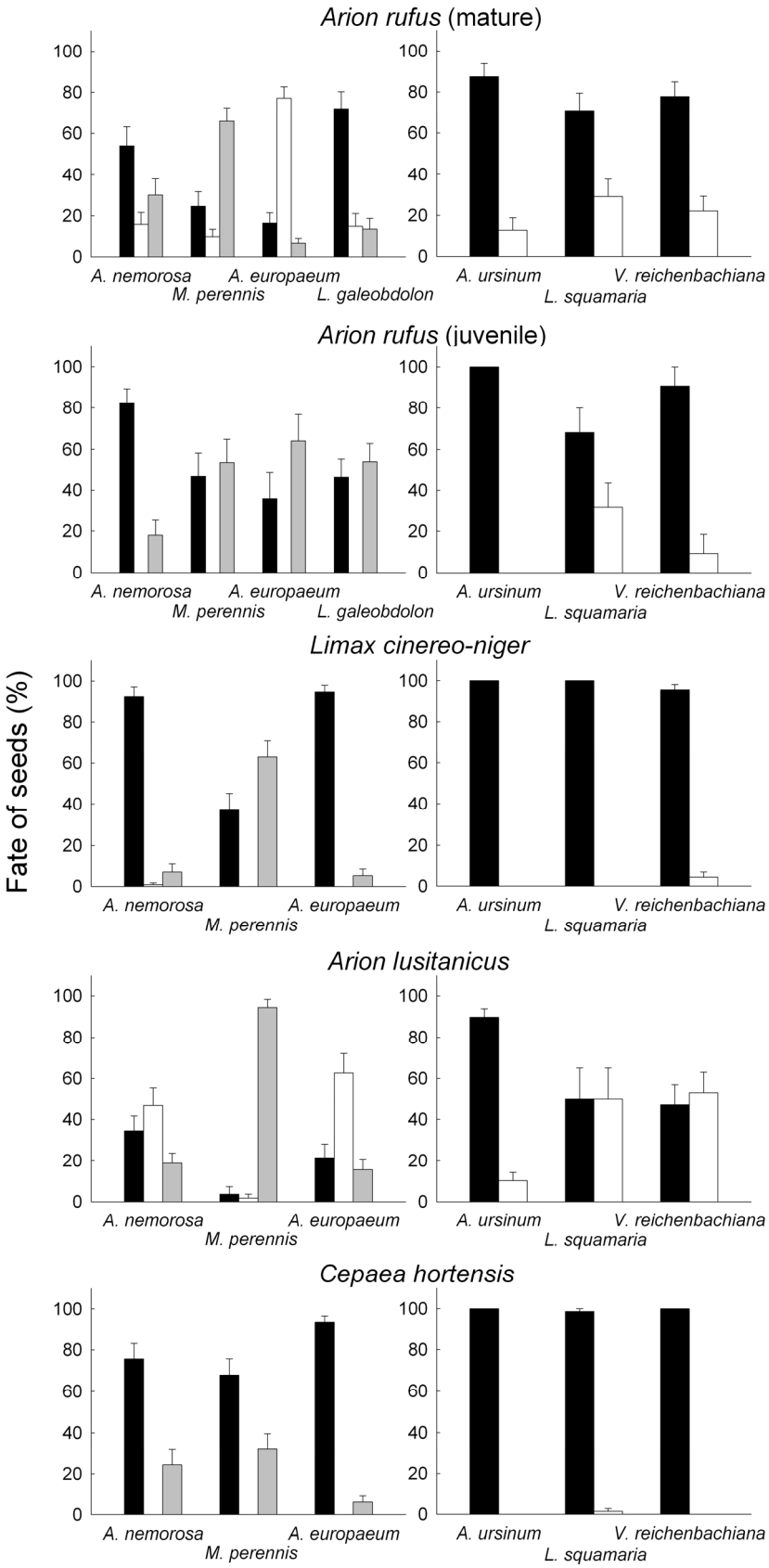

Figure 1. Fate of seeds offered to gastropods. Fate of seeds of myrmecochorous plants offered to slugs and snails. For *A. nemorosa, M. perennis, A. europaeum* and *L. galeobdolon* elaiosome damage was visible so that seeds were either swallowed (open bars), had their elaiosomes damaged by feeding (grey bars) or were not regarded (black bars). For *A. ursinum*, *L. squamaria and V. reichenbachiana* no elaiosome damage was visible and only the two categories swallowed and unregarded were distinguished. Results are given as mean + standard error (SE).

Discussion

Our study was intended to test the hypothesis that gastropods feed on seeds of myrmecochores and that they may serve as seed dispersers of these plants. Our results support this hypothesis as all gastropod species not only fed on seeds but also swallowed at least some of the seeds intact and could therefore act as seed dispersers. For *L. cinereo-niger* and *C. hortensis* the importance as seed dispersers is probably low as the number of individuals that swallowed seeds and the number of swallowed seeds were very small. Due to the differences found among the tested species it is likely that in a more complex species-rich gastropod community, some species will exclusively act as elaiosome predators or ignore seeds altogether while others may in fact disperse seeds. Interestingly, even a single species can have different roles and these roles may changes during ontogeny. Juvenile individuals of *A. rufus* swallowed seeds of small-seeded species but acted as elaiosome predators when seeds were large, whereas mature individuals swallowed a fair proportion of all seeds encountered. Thus, there may be a shift in the functional role of a species with age and size. While not tested, it is likely that other biotic and abiotic factors also influence the decision of a slug to swallow a seed or to feed on the elaiosome. While large seeded plants will be disadvantaged by a high abundance of small slugs, smaller seeded plants will not. The results of our study suggest that gastropod - plant interactions are highly complex in matters of seed ecology.

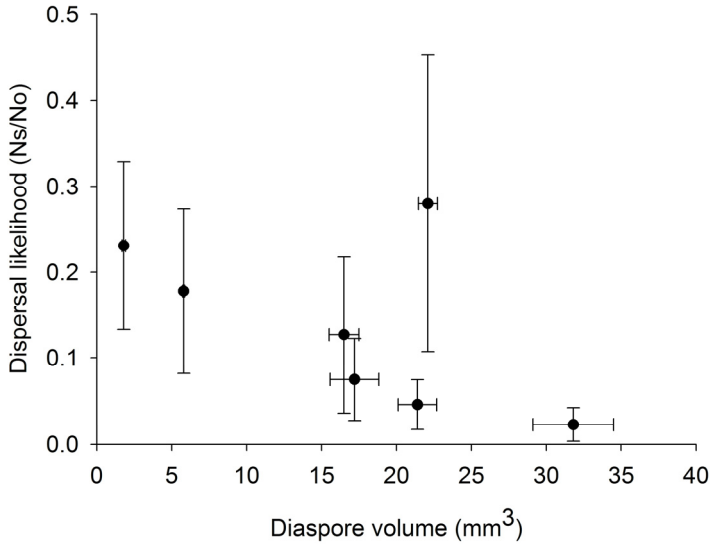

Figure 2. Relationship of diaspore volume and the dispersal potential. Relationship between diaspore volume and the dispersal potential of diaspores by gastropods described as the number of diaspores swallowed (Ns) of the number of diaspores offered (No) to all gastropod individuals. Results are given as mean ± standard error (SE).

The dispersal probability of seeds

Smaller seeds were more frequently swallowed than larger seeds. We assume that there are morphological restrictions preventing small gastropods swallowing large seeds which partly describe the pattern we found. As food items such as leaves are normally rasped by the radula of a gastropod, studies relating the size of food items to gastropod size would be helpful but we found none. It was shown for ants that diaspores with a high elaiosome-seed ratio were preferred in choice experiments (Gorb and Gorb 2003; Peters et al. 2003). Seeds of *A. europeaum* were swallowed by Arionid slugs far more frequently than all other seeds and this species also has the highest elaiosome-diaspore ratio (Table 2). This could probably explain why *A. europaeum* seeds deviate from the regression line of seed size and the proportion of swallowed seeds (Fig. 2).

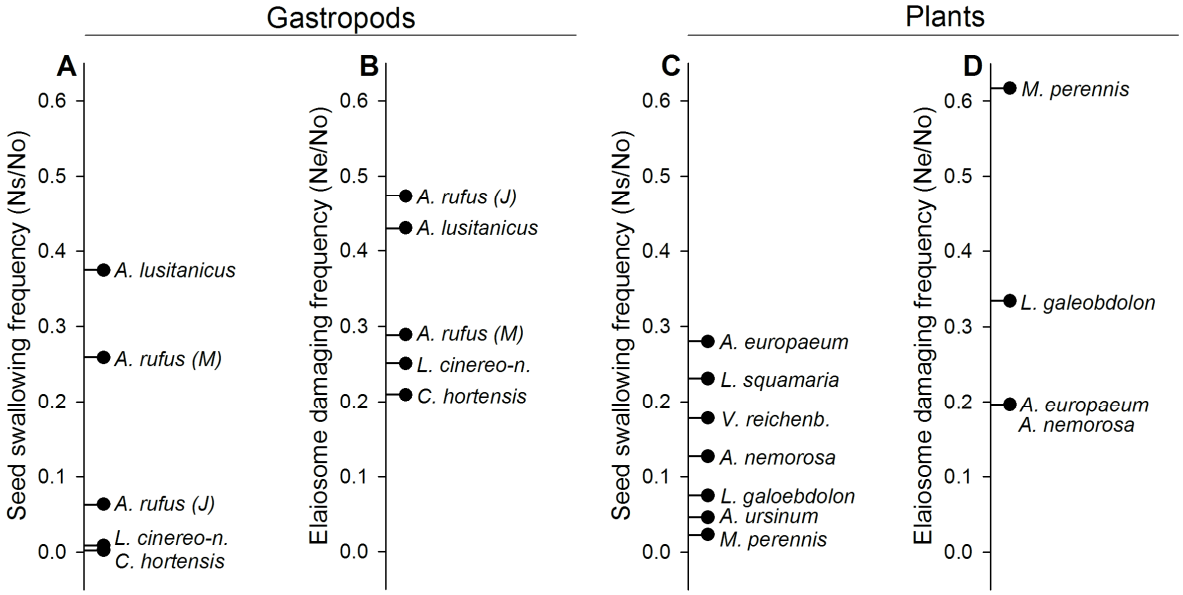

Figure 3. Ranking of gastropods and plants concerning the seed swallowing and the elaiosome damaging frequency. Ranking of the seed swallowing frequency of (A) gastropod species and (C) plant species and of the elaiosome damaging frequency of (B) gastropods and (D) pants. Swallowing of seeds is considered as the potential dispersal ability of gastropods. No, number of seeds offered to gastropods; Ns, number of seeds swallowed; Ne, number of seeds of which elaiosomes were consumed; M, mature individuals; J, juveniles.

With respect to the attractiveness of seeds to gastropods, it also has to be considered that the chemical content of the elaiosomes affects seed removal by ants (Boulay et al. 2006; Boulay et al. 2007b; Fischer et al. 2008; Gorb and Gorb 2003) and this could also influence

seed attractiveness to gastropods. *Asarum europaeum* is an obligatory myrmecochore which exclusively relies on animal vectors for seed dispersal, in contrast to the other plant species tested which may at least achieve short dispersal distances without seeds being transported by animals (Gorb and Gorb 2003; Sernander 1906; Ulbrich 1919). Such a means of dispersal selects for high attractiveness to seed dispersers and, *A. europaeum* seeds were often preferred to others if ants were given a choice (M. Türke, unpublished data; (Gorb and Gorb 2003)). Hence, the high attractiveness of *A. europeaum* seeds to Arionid slugs could be related to its chemical content as well. The seeds of *M. perennis*, the largest seeds in the set, suffered most elaiosome predation also of gastropod species that were generally more reluctant to feed on seeds, also suggesting a high attractiveness of these seeds to gastropods, but studies on its chemical content are not available.

Because the elaiosome resembles the insect hemolymph in its chemical composition the general trophic level of the gastropod species may also affect the likelihood of feeding on myrmecochorous seeds, i.e. predatory and omnivorous species should be more likely to consume such seeds (Türke et al. 2010). While most *Arion* species are omnivorous and were attracted to seeds in our study, *L. cinereo-niger* feeds on decaying plant material, algae and fungi (Anderson and MacMahon 2001; Bogon 1990; Falkner 1989; Frömmig 1950) and *C. hortensis* on young and senescent plants (Carter et al. 1979). Both of these species were less attracted to seeds.

Invasive versus native slug: a comparison

Seed dispersal mutualisms involving ants were shown to be susceptible to invasions of alien ant species with devastating effects on the distribution of the mutualistic plant species (Christian 2001; Traveset and Richardson 2006). The Spanish slug, *Arion lusitanicus*, has become an invasive slug in Central and Northern Europe. Currently, it is mostly found in open habitats where it is displacing the native large slugs *A. rufus* and *A. ater* (Fischer and Reischütz 1998; Kappes 2006; Kappes et al. 2009; Weidema 2006). We included *A. lusitanicus* in our study to compare its seed feeding behavior to that of *A. rufus*. We found no significant differences in the seed-feeding behavior. Both slugs were very similar in the proportions of seeds they swallowed of each species or the number of seeds of which elaiosomes were consumed. However, large seeded plants might suffer from the loss of the native slug. Arion *lusitanicus* is smaller (up to 15 cm) than *A. rufus* (sometimes exceeding 20 cm) and we found that juvenile *A. rufus* were not capable of swallowing larger seeds. Among the species we tested, the largest seeds were those of *M. perennis*. While six individuals of *A. rufus* swallowed a total of 12 seeds of this species, just a single individual of *A. lusitanicus* swallowed a single seed. Finally, we should furthermore consider that both species differ in

other means of behavior, which might impact on seed dispersal as well. But more studies comparing the movement and seed feeding behavior of both species are needed.

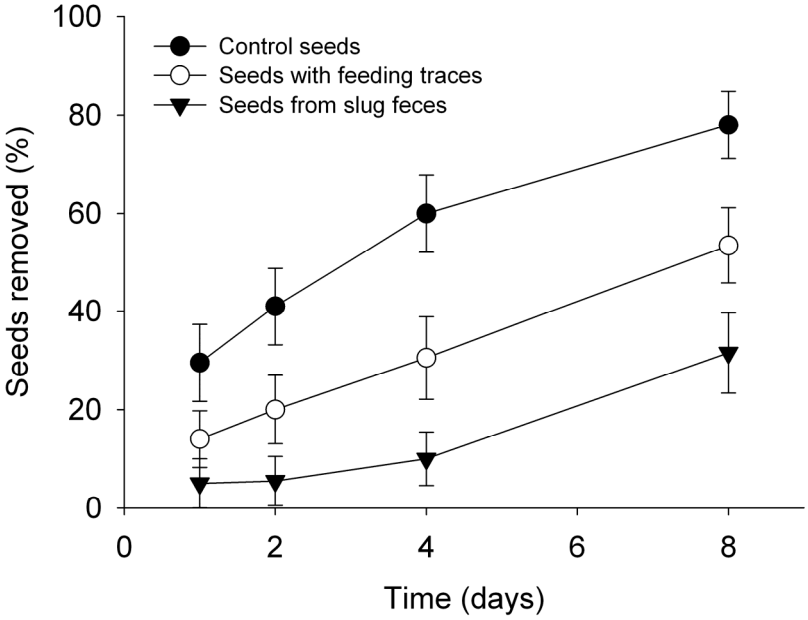

Figure 4. Field seed removal experiment with seeds fed on by slugs. Fate of seeds of *Anemone nemorosa* exposed in beech forests in the Hainich region (N=20 subplots). Seeds were either untreated (control), had feeding traces on the fruit skin inflicted by red slugs (*Arion rufus*) or had previously been swallowed and defecated by red slugs. Values are given as the mean±standard error (SE).

Gastropod effects on seed predation

The main seed predators of myrmecochorous forest herbs in Central Europe are rodents (Hulme 1998) although also ground beetles may destroy seeds of myrmecochores (Kjellsson 1985) or feed on the elaiosomes (Ohara and Higashi 1987). The predator-avoidance of seeds as a selective advantage of myrmecochory for the plants has been supported by most studies that addressed this hypothesis (summarized in (Giladi 2006)). In a laboratory study, it was suggested that slug-defecated seeds are less attractive to rodents than unconsumed seeds and are hence more likely to escape seed predation (Türke et al. 2010). In our experimental field study, seeds that were defecated by slugs or had feeding traces on the fruit skin of the achenes caused by slugs were much less likely to be removed from seed depots and this finding was consistent in managed and unmanaged forests. While we cannot differentiate between seed removal by certain animal taxa, visitation of seed depots by

gastropods was evident in most plots and their contribution to seed removal in the Hainich National Park was high (Türke et al. 2010).

Although the reduction in seed removal of seeds that had undergone gut passage in slugs might partly be due to a reduced attractiveness of these seeds to other gastropods, we also observed rodent traces in seed depots. Rodents (as indicated by opened achenes and rodent feces) consumed seeds unambiguously in some subplots, though they might have visited other depots without leaving traces, too, and they appeared to prefer untreated seeds to slug-consumed seeds or to locate them superior in some subplots: in each of two plot corners, nine control seeds were removed but only one seed with feeding traces in one corner and one defecated seed in the other corner, respectively. Despite the short distances between seed depots, rodents were not attracted to the seeds that had previously been fed on by slugs or couldn't locate them. On the other hand, we also found that in another subplot, where a mouse hole was located next to a depot with defecated seeds, all seeds except for two seeds from the three seed depots in the triangle in this corner were removed after one day already. In another plot corner where rodent traces were found, only two control seeds, four seeds with feeding traces and no defecated seeds were removed.

Slug feces themselves which coat defecated seeds might contribute to the repellent effect on seed predators. In addition, seed predators might have difficulties in locating seeds that slugs had fed on as the removal of elaiosomes has been shown to reduce the ability of rodents to find seed depots (Bond and Breytenbach 1985; Boyd 2001; Christian and Stanton 2004). In fact, while the elaisome helps rodents to find seeds, it is itself not attractive for consumption. In the chaparral shrub *Fremontodendron decumbens* in California, USA, rodents consumed the seed rather than the elaiosome (Boyd 2001). For several myrmecochores in temperate forests in West Virginia, USA, seeds were predated before dehiscence of capsules by rodents, yet elaiosomes were not consumed and left behind (Heithaus 1981). Thus, removal of the elaiosome is likely to reduce the frequency of finding seed depots by rodents but does not reduce attractiveness of seeds to rodents. For seeds swallowed by slugs, parts of or the whole elaiosome may still remain on seeds after gut passage, but sometimes the elaiosome is consumed partly before swallowing or breaks off from seeds during gut passage (Türke et al. 2010). Slug digestion could probably further reduce the content of fatty acids in elaiosomes and thus probably the intensity of their smell which can be used by rodents as an olfactory cue (Boyd 2001). However, studies on changes of the chemical content of slug-digested seeds or elaiosomes are still missing.

Interestingly, removal of seeds by gastropods was very fast and often occurred within one night after exposure. Most commonly seeds are regarded as having escaped predation when they were removed from seed depots by ants instead of seed predators (Bond and Slingsby 1984; Boulay et al. 2007a; Heithaus 1981; Manzaneda and Rey 2009). Thus,

swallowing of seeds by gastropods is likely to reduce pre-dispersal seed predation as well as post-dispersal seed predation.

Finally, consumption by slugs may also reduce seed predation because it reduces the aggregation of seeds which has been shown to enhance seed predation (Fenner and Thomson 2005). A reduction in seed aggregation by slugs is likely as seeds are dispersed by different individuals, moving in different directions and defecating ingested seeds over a period of time (Türke et al. 2010). Some ant species bury seeds inside their nests which makes it more difficult for rodents to locate the seeds (Giladi 2006; Heithaus 1981; Manzaneda and Rey 2009). We did not test whether slugs deposit seeds in buried locations. However, slugs often seek shelter for resting beneath leaf litter in the forest and may deposit seeds there and thus in some cases seeds might be less exposed when they have been dispersed by slugs compared to seeds that have been dropped by the plants. Inhumation is of great importance for seeds and besides ants also other invertebrates like Scarab beetles (Scarabeidae), ground beeltes (Carabidae), earthworms or snails could provide this service (Beattie and Culver 1982).

Conclusion

Our study has confirmed that gastropods feed on seeds of myrmecochores and are capable of dispersing a variety of seeds differing in size and shape. Gastropods may exhibit different functional traits for plants, either beneficial or detrimental, when dispersing seeds or consuming elaiosomes. Smaller seeded plants might benefit most from gastropodochory concerning the proportion of seeds dispersed. We found no major differences in the seed feeding habits of native *A. rufus* and invasive *A. lusitanicus*, but studies comparing the movement behavior of both species are still needed. The involvement of gastropods in seed ecology of myrmecochores makes this diffuse mutualism even more complex, but nevertheless we strongly encourage researchers to account for the seed dispersal by gastropods in their studies.

Acknowledgements

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CHAPTER FOUR

The Influence of Body Mass on the Seed Dispersal Potential of Slugs

Manfred Türke • Wolfgang W. Weisser

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Abstract

In a recent study, gastropods have been identified as seed dispersers of ant-dispersed plants (myrmecochores). Slugs swallowed diaspores entirely and defecated them intact and germinable or they only consumed the elaiosome, a nutrient-rich appendage on diaspores which consequently prevents dispersal. Juvenile red slugs (*Arion rufus*) did not swallow diaspores, while mature individuals did. However, seed feeding in mature slugs was also very variable. We investigated whether the seed feeding behavior of slugs might depend on body size. We collected 273 mature slugs in beech forests, assigned them to four body mass classes and offered 10 achenes of wood anemone (*Anemone nemorosa)* to each of 14 individuals per class. Slugs consumed the fruit skin of achenes (equivalent to the elaiosome) either by swallowing diaspores entirely or by rasping it with the radula. Larger individuals consumed more fruit skin and swallowed more diaspores than smaller ones (range 0-90% swallowed). The proportion of fruit skin consumed by swallowing seeds of the total fruit skin consumed increased with body mass, too. Thus, large slugs will more likely disperse diaspores. As slug populations of different forests differed in their body mass distribution, they might differ in their dispersal potential, too. Thus, we calculated the proportion of diaspores encountered by slugs that could theoretically be dispersed by the slug populations as the product of the proportion of diaspores swallowed per individual and body mass class and the proportion of individuals in each class per forest site, and this value ranged remarkably from 9 to 23 %.

Introduction

Plants adapted to seed dispersal by ants have undergone an incredible diversification due to the limited seed dispersal ability of ants (Lengyel et al. 2009). These so-called myrmecochores are often a significant part of a local plant community, accounting for as much as 50% of the plant species and more than 70% of emergent herbaceous stems (Beattie 1985). Myrmecochory is a global phenomenon and has evolved many times independently (Lengyel et al. 2009). The seed dispersal mutualistic network of ants and myrmecochores can be complex in many habitats. The diaspores of a particular plant species can be dispersed by several sympatric ant species which may exhibit different properties of seed dispersal related to dispersal distances and predator- or fire-avoidance (Beattie 1985; Gorb and Gorb 2003; Gorb and Gorb 1999; Ness et al. 2004; Pudlo et al. 1980). In addition, animals other than ants including rodents (Bond and Breytenbach 1985; Heithaus 1981), ground beetles and other insects (Ohara and Higashi 1987; Ohkawara et al. 1996) and slugs (Gunther and Lanza 1989; Mesler and Lu 1983; Türke et al. 2010) are also involved in the seed ecology of myrmecochores, predating on diaspores or elaiosomes, appendages found on all diaspores of myrmecochores, which are commonly fed on by the ants or their larvae (Gammans et al. 2005) and which contain nutrients such as fatty acids, amino acids, carbohydrates and vitamins (Fischer et al. 2008).

At recent times, gastropods have been demonstrated not only to predate on elaiosomes but also to disperse diaspores of myrmecochores (Türke et al. 2010). The authors found that slugs and snails were responsible for most diaspore removal of the myrmecochores wood anemone (*Anemone nemorosa* L.) and European wild ginger (*Asarum europaeum* L.) from experimental seed depots in an unmanaged beech forest in Central Germany. Moreover, the diaspore feeding behavior of slugs was confirmed in laboratory feeding experiments which revealed that seeds were defecated undamaged and germinable. Together with the observation that diaspores of different myrmecochorous species were defecated by slugs collected in beech forests, slugs must be considered as seed dispersers. Previous studies showed that gastropods disperse seeds of fleshy fruited plants (Gervais et al. 1998; Müller-Schneider 1967; Müller(-Schneider) 1934) and potentially also of non-myrmecochores such as the anemochore dandelion (*Taraxacum* agg.) (Honek et al. 2009) or gold-of-pleasure (*Camelina sativa*) (Kollmann and Bassin 2001). In myrmecochores, slugs are foraging for the elaiosome (Gunther and Lanza 1989; Mesler and Lu 1983; Türke et al. 2010). Slugs may swallow the diaspore entirely or they may exclusively consume the elaiosome by rasping it with their radula which will prevent dispersal of the diaspore (Türke et al. 2010). Thus, it is fundamental for the understanding of the seed dispersal mutualism of gastropods and myrmecochores to identify factors influencing this behavior.

The behavior and the morphology of ant species, in particular, body size and the span of the mandibles, influence which diaspores are dispersed and the distances they are transported (Boulay et al. 2007b; Gomez and Espadaler 1998b; Gomez et al. 2005; Gorb and Gorb 2003; Ness et al. 2004; Ness et al. 2009; Peters et al. 2003; Pudlo et al. 1980). Similar size-dependent dispersal ability may occur in slugs, too. While juvenile individuals of the red slug (*Arion rufus)* along with other smaller Arionid slugs did not swallow any diaspore of *A. nemorosa* or *A. europaeum* in laboratory feeding experiments, mature individuals did (Türke et al. 2010). This suggests that slug body size limits the size of diaspores that are dispersed. In this study, we investigated the role of body size for slug seed dispersal behavior, relating the proportion of diaspores swallowed to the body mass of *A. rufus* individuals.

Under the assumption that slug density, diaspore density and the probable diaspore encounters by slugs are constant between different forest sites and under the assumption that slugs behave the same in the field as in the laboratory with reference to the number of diaspores swallowed in relation to the number of diaspores encountered, we estimated and compared the proportion of diaspores that will theoretically be swallowed and dispersed by the whole mature *A. rufus* population at different forest sites.

Materials and methods

Study area

Field work and slug sampling were conducted in the Hainich-Dün region in Central Germany (10°47' E/ 51°15' N). The Hainich National Park and its surrounding areas are one of three regions in Germany where experimental plots for long-term biodiversity research have been established in the framework of the biodiversity exploratories project. Detailed information on the project design and on the region can be found in Fischer et al. (2010) and on the project's homepage (http://www.biodiversityexploratories.de/). Landscapes in the Hainich-Dün region comprise deciduous forests mostly dominated by European beech (*Fagus sylvatica*). Experimental plots are situated in forests of different management types, including age class forests (AF) where all trees in one stand are of the same age, selection-cutting forests (SF) where individual trees are harvested selectively and unmanaged beech forests (UF) in the Hainich National Park.

Diaspore material

Diaspores of wood anemone (*Anemone nemorosa* L.) were collected in the Hainich-Dün region on May 20th, 2009, and stored at 4° C until used in the experiment. The achenes

(small nuts containing a single seed) measure 4.2 – 5.0 × 1.8 - 2.4 mm including the slightly curved persistent style and are surrounded by a short-haired fruit skin (Bojňanský and Fargašová 2007). At the position where the peduncle was attached to the achene, before it was dropped from the plant, there are small remains of the peduncle which contain higher levels of lipid contents, and which, therefore, were denoted as the elaiosome (Canullo 1985; Ulbrich 1919). This part is, however, indistinct and not visible without dissection. Slugs are feeding on the fruit skin of the achene and they may do so by swallowing diaspores entirely or by rasping parts of or the entire fruit skin with the radula (Türke et al. 2010). The swallowing of the diaspore will lead to its endozoochorous dispersal, while feeding on the fruit skin without swallowing the diaspore doesn't. Previous experiments did not indicate that slugs were feeding preferentially on the fruit skin at the remains of the peduncle (Türke et al. 2010), where the elaiosome should be located (Canullo 1985; Ulbrich 1919). Thus, we did not differentiate feeding by the location on the achene and regarded all feeding as some kind of elaiosome consumption. Concerning ants, diaspores were collected in some experiments (Canullo 1985; Sernander 1906), but were unattractive to ants in some others (Sernander 1906; Türke et al. 2010).

Slug size and seed dispersal potential

While juvenile red slugs (*Arion rufus* L.) were not capable of swallowing diaspores of *A. nemorosa*, mature individuals were (Türke et al. 2010). However, there were considerable differences in the feeding behavior of mature red slugs, according to the proportion of seeds that were swallowed or of which only the fruit skin was rasped (Türke et al. 2010). Thus, we suggested that the likelihood of seed swallowing behavior is dependent on body size.

Mature individuals measure 10 to 15 cm, but they may sometimes reach up to 20 cm body length (Bogon 1990). We collected 273 supposedly mature individuals (>10 cm body length) of *A. rufus* at seven different sites in beech forests in the Hainich region on May 19th and 20th, 2009, a time at which *A. nemorosa* sheds its diaspores. Forests differed in management types (UF = 2 plots, SF = 2 plots and AF = 3 plots). All mature individuals of *A. rufus* that we discovered were collected at each forest site. The size of the area searched for slugs varied as slug abundance also varied between sites. Slugs were brought to the laboratory on May 20th, 2009 and the live body mass was measured with a Monobloc inside balance (Mettler-Toledo GmbH; accuracy 0,001 g). Mean body mass was 11.0±3.5 g (mean±SD), ranging 3.9 g to 24.0 g.

All individuals were assigned to four body mass classes: (1) the minimum to the 25% quartile of body mass of all individuals (3.9 - 8.6 g), (2) more than the 25% quartile to the median (8.7 - 10.9 g), (3) more than the median to the 75% quartile (11.0 - 13.2 g) and (4) more than the 75% quartile to the maximum (13.3 - 24.0 g). We randomly selected 14

individuals of each group and kept them singly in plastic fauna boxes measuring 27 × 18 × 17 cm (length, width, height) (Savic Fauna box, 6 L; http://www.savic.be) provided with moist paper towels. The individuals were fed with wild herbs (mainly dandelion (*Taraxacum* ssp.) leaves) for three days, and then they were starved for another three days (except for the wet paper in the box on which individuals fed frequently). Then we offered 3 g of potato and 10 diaspores of *A. nemorosa* to each slug. After 48 hours we assessed the number of diaspores swallowed and for all remaining diaspores the proportion of fruit skin area that was consumed. Swallowed diaspores have 100% of their fruit skin consumed. The fruit skin area consumed per diaspore was summed over all diaspores for each individual and then we calculated the proportion of fruit skin area consumed of the total fruit skin of all 10 diaspores.

Statistics

Statistical analyses were performed using R 2.8.0 (R Development Core Team; http://www.r-project.org/). For the comparison of slug body mass between forest sites, we performed an ANOVA with the function “aov” and the site defined as fixed factor. We performed a Fisher’s exact test for the comparison of the number of individuals of the four body mass classes that swallowed diaspores using the function “fisher.test”. We further tested for a correlation (Spearman’s Rank Correlation with the function “cor.test” and the method=”spearman”) of slug body mass with the number of diaspores swallowed, the proportion of total fruit skin area consumed of all ten diaspores and with the proportion of fruit skin consumed by swallowing diaspores of the total fruit skin area consumed of all ten diaspores.

Results

Slug size and diaspore feeding behavior

Slugs differed significantly in body mass in relation to the forest site where they were collected (ANOVA; $F_{6,264}$= 8.39, p<0.001). Slug individuals swallowed 1.7±0.3 diaspores (mean±SE) and consumed 31±3 % of the total fruit skin area of all ten diaspores (including swallowed seeds; mean±SE). Just a single individual did not consume fruit skin of any diaspore. The number of slug individuals that swallowed diaspores increased with higher body mass classes (Fisher’s exact test; p<0.001; Table 1). Larger slugs significantly swallowed more diaspores than smaller individuals (Spearman’s Rank Correlation; N=56, rho=0.60, p<0.001; Fig. 1 A; Table 1), consumed more fruit skin (N=56, rho=0.44, p<0.001; Fig. 1 B; Table 1) and they also consumed more fruit skin by swallowing seeds in relation to the total fruit skin consumed of all ten diaspores (N=55, rho=0.66, p<0.001; Fig. 1 C).

Table 1 Number of swallowed diaspores (mean±SE) and proportion of total fruit skin area consumed of all 10 diaspores (mean±SE) of *A. nemorosa* by *A. rufus* individuals within the four body mass classes.

	Body mass classes			
	Class 1	Class 2	Class 3	Class 4
Range of body mass (g)	3.9 - 8.6	8.7 - 10.9	11.0 - 13.2	13.3 - 24.0
Individuals that swallowed seeds	4	7	9	14
Number of seeds swallowed	0.4±0.2	1.1±0.4	1.5±0.4	3.8±0.7
Fruit skin area consumed	19±4	27±5	31±5	47±6

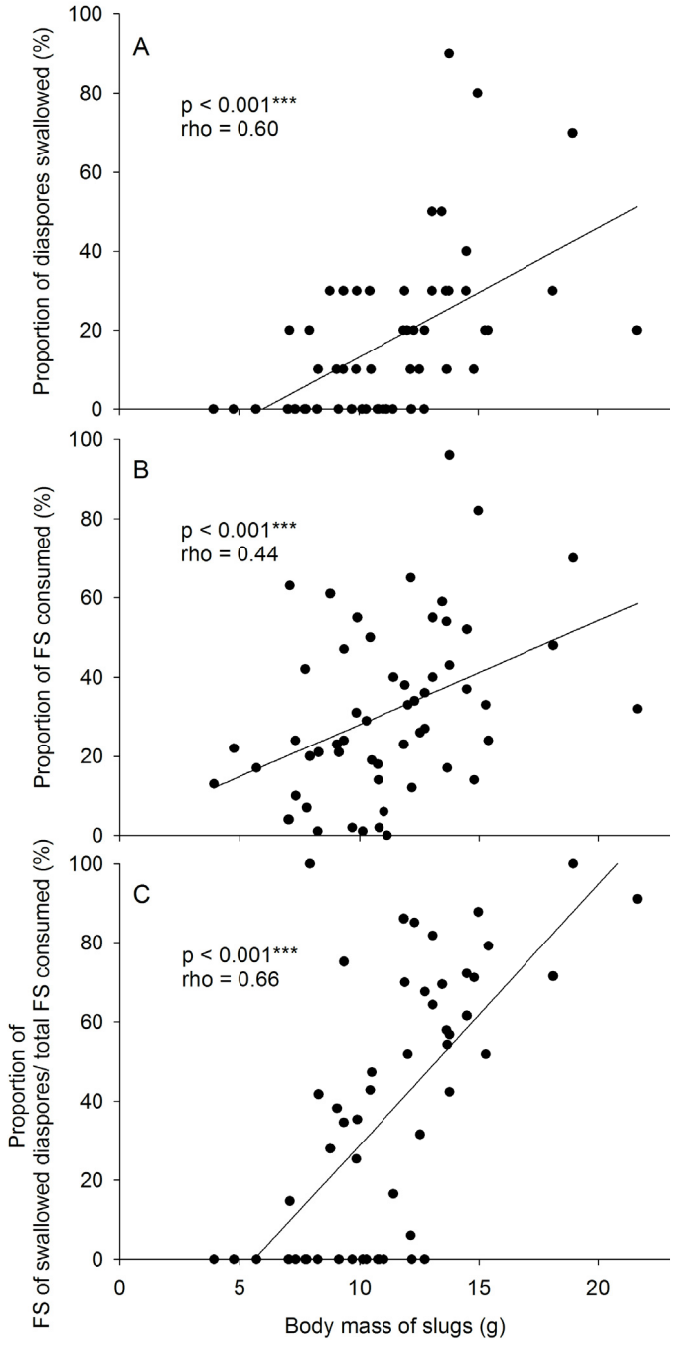

Figure 1 Relationship of slug body mass and (A) the number of diaspores swallowed, (B) the proportion of diaspore fruit skin area (FS) consumed (a swallowed diaspore was regarded as if 100% of its fruit skin was consumed) and (C) the proportion of fruit skin (FS) that was consumed by swallowing diaspores of the total fruit skin area consumed of all ten diaspores. rho, Spearman's Rank Correlation Coefficient; p, p-value of the correlation.

Dispersal potential of local slug populations

We calculated the mean proportion of diaspores swallowed per individual in each body mass class (Table 1). And for each forest site, we calculated the proportion of individuals in each body mass class (Table 2). The sum of the product of both terms over all body mass classes gives the proportion of diaspores that could potentially be dispersed by the local slug population. In average, 17±2 % (mean±SE) of the *A. nemorosa* diaspores encountered by mature red slugs could be dispersed and these proportions range remarkably from 9 to 23 % between different sites (Table 2).

Table 2 Distribution of slug individuals over four body mass classes collected at seven forest sites and the proportion of A. nemorosa diaspores that could theoretically be dispersed by the local slug population, based on the results of the diaspore feeding experiment.

	Body mass classes				
	Class 1	Class 2	Class 3	Class 4	
Forest site	Proportion of individuals in body mass classes (%)				Proportion of seeds dispersed in forest site (%)
AF 1	45	30	0	25	15
AF 2	18	19	27	35	20
AF 3	15	27	31	27	18
SF 1	11	11	33	44	23
SF 2	36	30	19	15	13
UF 1	10	29	33	29	19
UF 2	54	22	22	3	9

NOTE: AF=Age class forest, SF=Selection-cutting forest, UF=Unmanaged forest

Discussion

Seed dispersal of myrmecochorous forest herbs by gastropods has only recently been described (Türke et al. 2010). However, only little is known about the mechanistic aspects that influence the behavior of a slug and that determine whether a diaspore is swallowed entirely or the elaiosome is consumed. Only swallowed seeds will be dispersed by the slug. We demonstrated that the body size of individuals of *A. rufus* is an important factor in the diaspore feeding behavior of a slug.

Seed dispersal frequency in relation to slug size

Diaspores that are swallowed will be dispersed by the slug, while diaspores of which only the elaiosome is consumed will not be dispersed. Diaspores with removed elaiosome are also less likely to be dispersed by ants (Bond and Breytenbach 1985; Gorb and Gorb 2003; Sernander 1906) and thus elaiosome predation by slugs will prevent dispersal of diaspores by ants if both taxa are present. Ground beetles, for instance, consumed the elaiosomes of 85% of *Trillium* diaspores in a temperate, deciduous forest in Japan, inhibiting dispersal by ants (Ohara and Higashi 1987). Whether slugs swallow diaspores which have their elaiosome removed has still to be tested. In our study, it emerged that (1) more large slugs swallowed diaspores than smaller slugs and (2) slugs with greater body mass swallowed more diaspores than smaller individuals (also in relation to the total fruit skin area consumed of all ten diaspores). Thus, large slugs are more effective seed dispersers of diaspores of *A. nemorosa* than small individuals, which will mainly act as elaiosome predators by rasping the fruit skin of the achene without moving it.

While in our study individuals of *A. rufus* of the smallest body mass class swallowed diaspores, Türke et al. (2010) found that juvenile individuals of *A. rufus* with less than 8 cm body length were exclusively acting as elaiosome predators of *A. nemorosa* and *Asarum europaeum*. However, 80% of myrmecochorous diaspores in European mesic habitats range 1-3 mm in size and are often much smaller than those of *A. nemorosa* (Servigne 2008). Juvenile *A. rufus* can disperse diaspores which are smaller than those of *A. nemorosa* (Türke & Weisser, unpublished manuscript) and may be effective seed dispersers for these species. We can expect that for small diaspores, again, the body size of small slugs influences the number of diaspores swallowed. Interestingly, the body size of ants also influences the seed dispersal behavior and the effectiveness of dispersal (Gomez and Espadaler 1998b; Gorb and Gorb 2003; Ness et al. 2004).

There are many other possible factors besides body size that influence whether a slug swallows a diaspore or consumes the elaiosome. Prospective research on this topic should address the following questions: Do biotic factors such as hunger influence the motivation to swallow diaspores? Could abiotic factors like humidity or temperature influence the diaspore feeding behavior of slugs? Does diaspore size determine the swallowing of diaspores? As swallowing a diaspore takes less time than consuming the elaiosome (M. Türke, personal observations), could competition among conspecifics or with interspecific competitors force slugs to accelerate diaspore consumption by swallowing diaspores? Many more factors influencing the seed dispersal potential of slugs are imaginable and have to be tested consecutively.

Influence of the habitat on seed dispersal

We found that there were significant differences in body mass between individuals of *A. rufus* sampled in different forests in the same region. When we applied the proportion of diaspores swallowed by individuals in the four different body mass classes in the laboratory to the body mass distribution of slugs at the different forest sites, we found a great divergence between sites in the proportion of diaspores that could theoretically be swallowed and dispersed by slugs.

There is a high variability of body mass within individuals of the same age of the leopard slug (*Limax maximus* L.) and the banana slug (*Ariolimax columbianus* Gould*)* (Pearson et al. 2006; Prior 1983). (Lyth 1983) demonstrated that the water content of slugs including the black slug (*Arion ater* L.*)*, the sibling species to *A. rufus,* was constant between sites and was independent of the soil water content. Thus, other parameters influencing the body mass and size of a slug must be related to the forest sites. The factors influencing the body mass can possibly vary, including the nutritional value of food and related assimilation rates in slugs (Davidson 1976), interspecific competition for food (Rollo 1983) or parasitism (Baur and Baur 2005; Cagáň and Shoaib 2003; Shoaib and Cagáň 2004; Speiser et al. 2001). In this way, habitat parameters can impact on slug body size and probably also on the seed dispersal potential of the whole slug population.

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CHAPTER FIVE

Are Gastropods, rather than Ants, Important Seed Dispersers of Myrmecochorous Forest Herbs?

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Abstract

Seed dispersal by ants (myrmecochory) is widespread and seed adaptations to myrmecochory are common, especially in the form of fatty appendices (elaiosomes). In some habitats, however, dispersal of myrmecochorous seeds is thought to be ant-limited. In a recent study, slugs were identified as seed dispersers of myrmecochores in a Central European beech forest. Here we used 105 beech forest plots to test whether myrmecochore presence and abundance is related to ant or to gastropod abundance and whether experimentally exposed seeds are removed by gastropods. Myrmecochorous plant cover was positively related to gastropod abundance, but negatively to ant abundance. Gastropods were responsible for most seed removal and elaiosome damage, while insects (and rodents) played minor roles. These gastropod effects on seeds were independent of region or forest management. We suggest that these terrestrial gastropods can generally act as seed dispersers of myrmecochorous plants or even substitute myrmecochory.

Introduction

Ant dispersal of seeds has strongly promoted plant diversification in angiosperms (Lengyel et al. 2009) and at least 11,000 plant species from 77 families worldwide are thought to be adapted to seed dispersal by ants (Lengyel et al. 2010), many by means of a nutrient-rich seed appendage, the elaiosome (Sernander 1906). These so-called myrmecochores are found almost throughout the world (Beattie 1985; Lengyel et al. 2010) and dominate plant communities in many habitats, both in terms of species richness and abundance (Sernander 1906; Ulbrich 1919; Handel et al. 1981). In a seminal study correlating the abundance and species richness of myrmecochores with the abundance of ants in 10 different forest types of West Virginia, USA, Beattie and Culver (1981) found that ant abundance was generally positively correlated with the number of myrmecochorous species in these forests. There were, however, exceptions to this general pattern involving forests of high myrmecochore abundance but low abundance or even absence of ants. Similarly, in Central Europe a diverse community of myrmecochores can be found in forests dominated by European beech (*Fagus sylvatica* L.), including some highly abundant species such as wood anemone (*Anemone nemorosa* L.) or bear's garlic (*Allium ursinum* L.), whereas ants can be rare and of low species diversity compared with other forest habitats (Seifert 1986; Wlodarczyk 2010). This suggests that seed dispersal of myrmecochores in European beech forests may well be ant-limited.

In Europe, due to their limited dispersal distances many forest myrmecochores are used as indicator plants of ancient woodlands (Wulf 1997; Orczewska 2010). However, recently such myrmecochores were found to colonize forests rapidly, even though both unassisted seed movement and vegetative spread are very limited among these species (Brunet and von Oheimb 1998a; Verheyen and Hermy 2001; Orczewska 2009). This clearly suggests that animal vectors do transport diaspores of forest myrmecochores. If ants are rare, other animals must be myrmecochore seed dispersers in beech forests. Among other taxa, rodents and insects other than ants are known to be seed predators of myrmecochores but are unlikely to disperse their seeds (Heithaus 1981; Ohara and Higashi 1987).

Recently, several of us (Türke et al. 2010) demonstrated that terrestrial gastropods consumed seeds of the two myrmecochores, wood anemone and European wild ginger (*Asarum europaeum*), in an unmanaged beech forest in Central Germany and that seed removal rates by gastropods were higher than those by insects or rodents. In the laboratory, some slug species either consumed elaiosomes or swallowed seeds entirely, however, without affecting the viability of defecated seeds (Türke et al. 2010). Thus, gastropods appear to act as seed dispersers rather than as seed predators. Moreover, red slugs (*Arion rufus*) may disperse seeds further than ants do in average (Gomez and Espadaler 1998;

Türke et al. 2010). In conclusion, gastropods may well be important seed dispersers of forest myrmecochores.

We tested two hypotheses on seed dispersal of myrmecochores in beech forests. First, we suggest a discrepancy in the abundance of myrmecochores and ants in beech forests. We therefore conducted vegetation and ant abundance surveys in 105 beech forest plots of varying management types. Plots were distributed over three German regions with a maximum distance of 635 km between sites. Second, we suggest that gastropods disperse a majority of seeds of myrmecochores in beech forests. To test this, we exposed seeds of two abundant myrmecochores, wood anemone and European wild ginger, in 47 beech forest plots where we differentially excluded rodents, gastropods, and insects.

Materials and Methods

Study areas

The study was conducted in three German regions within the framework of the Biodiversity Exploratories project (www.biodiversity-exploratories.de, Fischer et al. 2010), investigating the relationship between land use and biodiversity: (1) the UNESCO Biosphere Reserve Schorfheide-Chorin in North-eastern Germany (NE region) (13°76' E/ 53°00' N), (2) the National Park Hainich and its surrounding areas (Hainich-Dün) in Central Germany (Central region) (10°47' E/ 51°15' N), and (3) the UNESCO Biosphere Area Schwäbische Alb (Swabian Jura) in South-western Germany (SW region) (9°39' E/ 48°43' N). The exploratories comprise 150 100 × 100 m experimental forest plots of varying management types. We selected the 105 forest plots that were dominated by European beech (*Fagus sylvatica* L.) including different management types (for details see Online Appendix A: Extended methods and results, table A1).

Species involved

European beech is a commercially and ecologically important tree species, widely distributed over large parts of Europe and the most common and on favourable sites dominant deciduous tree in Germany (Czajkowski et al. 2006). A great number of the herb species in mature beech forests are thought to be myrmecochores (ant-dispersed plants) (Sernander 1906; Ulbrich 1919). In our seed removal experiment, we used diaspores (referred to as seeds in the following) of two myrmecochorous spring geophytes, wood anemone *(Anemone nemorosa* L.) and European wild ginger (*Asarum europaeum* L.). Seeds of both plants bear an elaiosome (Servigne 2008), though it is rather indistinct in wood anemone. Wood anemone forms dense carpets in forest plots in the Central and SW regions but is less

abundant in the NE region. Wild ginger is also common in plots in the Cental and SW regions, but was not recorded in the NE region. Ant nest densities in mature beech forests are often low and can mostly be attributed to the red ant (*Myrmica ruginodis* Nyl) and the arboreal brown tree ant (*Lasius brunneus* Latreille) (Seifert 1986; Wlodarczyk 2010). In contrast, beech forests harbor a diverse fauna of terrestrial gastropods (Müller et al. 2005), including a number of species that are large enough to potentially disperse seeds endozoochorously. Among these species, the abundant red slug (*Arion rufus* L.), growing 10-20 cm, was described as a seed disperser of wood anemone and wild ginger (Türke et al. 2010). Therefore, we focused on this species in our observational slug survey. However, we cannot exclude or even suggest that other gastropods may disperse the seeds of myrmecochores, too, but feeding experiments are still missing for most species.

Vegetation, ant and gastropod surveys

In 2009, we recorded vegetation twice (in spring and summer/autumn) in a 20 × 20 m core area of each plot. We identified all herbaceous vascular plants (excluding phanerophyte seedlings) and estimated their percentage cover. We classified species as ant-dispersed according to Servigne's (2008) list of 260 European ant-dispersed plant species.

In 2008, ants and gastropods were sampled using three pitfall traps of 15 cm diameter with a funnel per plot (see Lange et al. 2010 for general trap design). On all plots, samples were collected monthly from April to October. Ants and gastropods were counted in 10 to 18 samples (11.5±0.3 mean±SE) per plot and 1211 samples in total. In some plots less than 10 samples were sorted due to failures mostly caused by traps being destroyed by large mammals. As pitfall traps provide evidence for the presence of seed-dispersing ants in the plot, but not for their abundances because ants may recruit their nestmates by pheromone trails to the trap, we used the proportion of pitfall trap samples with ants as a measure of ant abundance. Species of ants or gastropods were not identified.

Seed removal experiment

For our seed removal experiment, we collected ripe seeds of wood anemone and wild ginger in the Central region and in deciduous forests around Jena, respectively, in May and June 2009. Seeds were stored at -20° C until the start of the experiment to prevent desiccation of elaiosomes. The seed removal experiment was carried out in all regions representing different management types in each region (total N=47 plots, Online Appendix A, table A1). In each plot we exposed seeds in batches of ten on 10 × 10 cm wooden plates (seed depot), sheltered against rain by a 12.5 × 12.5 cm plastic roof supported by toothpicks. Seeds dropped by plants are exposed on the forest floor similar to our seed depots. We offered

seeds while our study species were fruiting, for wood anemone we exposed seeds from 7 May to 11 June 2009 and for wild ginger from 29 June to 25 July 2009.

We applied four animal exclusion treatments (as described in Türke et al. 2010): (1) unlimited access to all animals (control): seeds were not protected against animals, (2) access to rodents and insects (gastropod exclusion): the wooden plate was outlined with 1 × 1 cm slug repellent paste (IRKA Schneckenabwehrpaste, R + M Gartenbedarf GbR); in four directions of the seed depot, 18 cm long bridges made of zinc coated 2.8 mm strong wire connected the forest floor with the centre of the seed depot, allowing access for ants and other small insects. Rodents could enter either, (3) access to gastropods and insects (rodent exclusion): seed depots were covered by a 11 × 11 × 4 cm metal cage with a mesh size of 28 × 10 mm (rhombus-shaped mesh) that excluded only rodents and (4) access to insects (gastropod and rodent exclusion): as in (2) but the seed depot was additionally covered by a cage and ant bridges led through the cage mesh, allowing only insects to enter the seed depot.

Effectiveness of the animal exclusion techniques and the efficacy of slug repellent on slugs, rodents and ants were tested in preliminary experiments (Appendixes S5–S8 in Türke et al. 2010). We additionally evaluated their influence on arthropods in the field and on ants in the laboratory by video recordings (Online Appendix A). In the field, some seeds were removed by slugs (for an example video of *A. rufus* swallowing *A. europaeum* seeds see Online Appendix B: slug swallowing seeds.mpg) but not a single ant was observed. Based on the video recordings in the field, we conclude that our methods might underestimate elaiosome damage by arthropods (Online Appendix A, table A2) but not seed removal: elaiosome-feeding arthropods visited depots with slug repellent less often, but seed-removing arthropods (carabids) removed similar numbers of seeds from depots with or without slug repellent (for an example video see Online Appendix C: Carabid removing seed.mpg). In the laboratory, ants removed most seeds of wild ginger from depots protected by slug repellent over the bridges (for an example video of the black-backed meadow ant (*Formica pratensis* Retzius) see Online Appendix D: ant removing seed.mpg) but no seeds of wood anemone were removed or elaiosomes consumed by ants.

The different treatments were applied to seed depots in the corners of two 4 × 4 m squares (subplots) per plot, which were situated in two opposite corners of each 100 × 100 m plot. Thus, in total we exposed 752 seed depots (47 plots × 2 subplots × 4 treatments × 2 plant species). Three days after exposure we counted the remaining seeds and recorded feeding traces on seeds or elaiosomes. Eleven seed depots were destroyed by animals, most likely by wild boars and had to be excluded from the analysis. We also excluded 19 seed depots where gastropod exclusion had failed, as indicated by mucus. Therefore, we analyzed data from 722 seed depots.

We additionally counted all red slugs we observed, walking a 10 × 10 m area in five transect lines with the subplot in its centre. During the wood anemone experiment we counted slugs on four consecutive days in each subplot and during the wild ginger experiment we counted two times with two days delay between both surveys. Sampled individuals in each region were dissected for species identification.

Statistical analysis

Statistical analyses were performed using R 2.8.0 (R Development Core Team 2011). We tested for a correlation of ant or gastropod abundance and the cover or species number of herbs or myrmecochores with Spearman's rank correlation. In field seed removal experiments, we analyzed variation in the number of removed seeds and in the sum of removed seeds and remaining seeds with consumed elaiosomes between different treatments in a linear mixed-effects model (fit by the Laplace approximation for binomial errors) using the function ''lmer'' in the lme4 package. Plant species, regions and treatments were treated as fixed effects and plots and subplots nested in plots were treated as random factors. Multiple comparisons of means (Tukey contrasts) were performed using the function ''glht'' in the multcomp package. In addition, for each exploratory we tested the effect of management types on seed removal. Means and standard errors of variables are given throughout.

Results

Myrmecochore and seed disperser relationship

We recorded 231 herb species in the 105 plots of which 46 were classified as myrmecochores. The number of myrmecochores increased with the number of herb species (Spearman's rank correlation; rho=0.79, $p<0.001$, N=105). On average, 20.3±1.2 herb species (range 0–73 species) were observed per 20 × 20 m with a mean cover of 29±3%, including 5.6±0.3 myrmecochorous species (range 0–15 species) with a mean cover of 12±1%. Myrmecochore cover increased with the number of myrmecochorous species (rho=0.74, $p<0.001$).

On average, 9±4 ant individuals were trapped per pitfall trap. Ants were trapped in 44±3% of the traps per plot, ranging from 0% (11 plots) to 100% (11 plots). The proportion of pitfall-trap samples with ants, i.e. our measure of ant abundance, was independent of the cover of all herbs (Spearman's rank correlation; rho=-0.15, p=0.12) and the total number of herb species (rho=-0.05, p=0.59). However, it was significantly negatively – rather than positively –

correlated with the cover of myrmecochores in plots (rho=-0.33, p<0.001, fig. 1 *A*) and the number of myrmecochorous species (rho=-0.26, p=0.007, fig. 1 *B*).

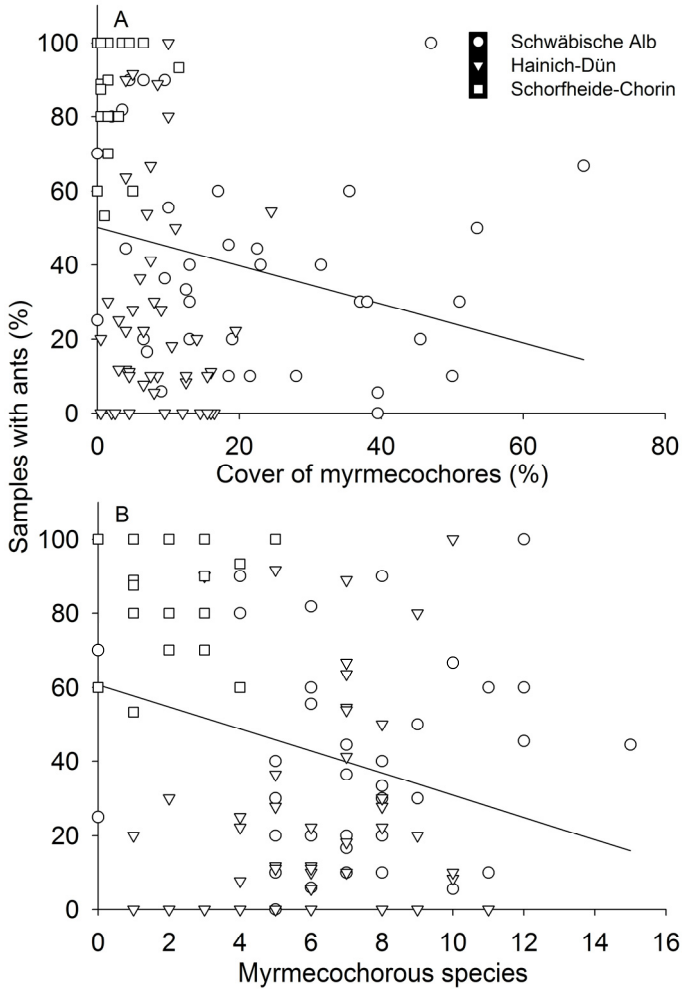

Figure 1: Relationship of the abundance of ants in beech forests indicated by the proportion of pitfall trap samples containing ants and (A) the cover of myrmecochores and (B) the number of myrmecochorous species (N=105 plots). Contrary to expectation, myrmecochorous herbs in beech forests increase in abundance and diversity as ant density decreases. Least-squares regression lines are given to suggest the trends.

The mean number of gastropods per pitfall trap sample (7.9±0.6; range 0.9-29.7 individuals/sample*plot) was significantly positively correlated with the cover of myrmecochores (rho=0.42, p<0.001, fig. 2 *A*) and of all herb species (rho=0.33, p<0.001), and with the number of myrmecochorous (rho=0.25, p=0.011, fig. 2 *B*) and all herb species (rho=0.23, p=0.020).

Seed removal experiment

In total, 26% of all seeds were removed from depots, fewer of wood anemone (598 seeds; 17%) than of wild ginger (1,299 seeds; 35%; GLMM; z=-20.53, p<0.001, fig. 3). 10% of the seeds that remained in depots had feeding traces on elaiosomes, again fewer of wood anemone (109 seeds; 3%) than of wild ginger (631 seeds; 17%; GLMM for the sum of

removed seeds and remaining seeds with elaiosome damage; z=-32.26, p<0.001). Red slugs were observed in 85% of the plots at the time of the experiment, ranging from none up to 15 specimens (0.78±0.04) per 100m^2, and 48% of the seed depots without restricted entrance for gastropods had unambiguously been visited by gastropods as indicated by mucus or feces. Within seed depots, we recorded 78 snails and 39 slugs including 29 red slugs.

Seed removal differed significantly between treatments (GLMM with Tukey post-hoc tests; p<0.001; fig. 3): most seeds were removed in controls, followed by treatments to which gastropods and insects had access. There was no difference between teatments with access to rodents and insects (gastropod exclusion) and with access to insects (gastropod and rodent exclusion) (z=1.27; p=0.57). Insects contributed only marginally to seed removal and elaiosome damage. Likewise, the impact of rodents on seeds was negligible. In contrast, gastropods were responsible for most of the seed removal and elaiosome damages in both plant species. The number of removed seeds differed significantly among regions and, in the NE region, also between forest management types (Online Appendix A, table A2). However, there were generally no differences between treatments among regions and management types (Online Appendix A, table A2).

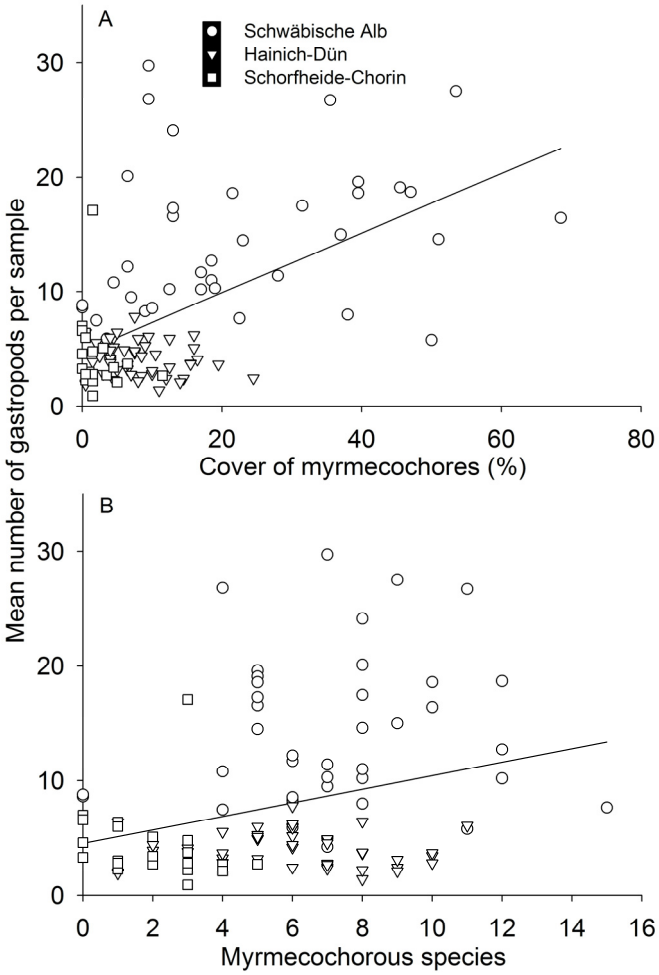

Figure 2: Relationship of the abundance of gastropods in beech forests indicated by the mean number of gastropods per pitfall trap sample and (A) the cover of myrmecochores and (B) the number of myrmecochorous species (N=105 plots). More gastropods were trapped in plots with higher myrmecochore abundance and species richness. Least-squares regression lines are given to suggest the trends.

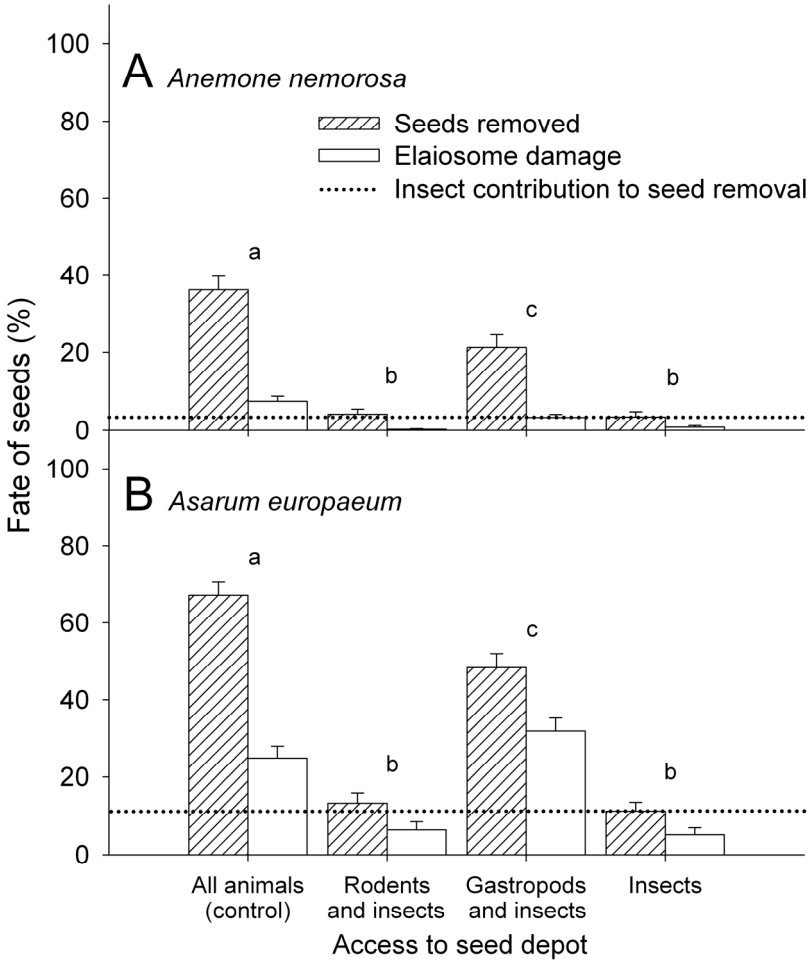

Figure 3: Fate of seeds of (A) *Anemone nemorosa* and (B) *Asarum europaeum* exposed in 47 beech forest plots with restricted access of certain animal taxa. Notice the negligible contribution by insects and the major role played by gastropods. Values are given as the mean±standard error (SE). Results of a GLMM (Tukey contrasts) are shown for comparisons of treatments; treatments with different letters differ significantly at $p<0.001$.

Discussion

For several reasons, our results suggest that dispersal by gastropods may be common among myrmecochores in Central European beech forests. Firstly, ant and myrmecochore abundance were negatively related to each other (fig. 1). Mature shaded beech forests are rather moist and cool while most ants prefer warm habitats (Seifert 2007). Only a few ant species exist in shaded beech forests (Seifert 1986; Wlodarczyk 2010). Moreover, their nest densities are low. In contrast, some very common myrmecochores in beech forests such as wood anemone or bear's garlic depend on moisture (typical for most mature beech forests), occur at high densities on the forest floor and produce many seeds per square meter (Tutin 1957; Shirreffs 1985). Thus, at least in European beech forests, habitat requirements of ants and myrmecochores differ profoundly.

Second, myrmecochores in beech forests inevitably require animal vectors for dispersal. The mean dispersal distance of seeds of wood anemone without animal vector is estimated to be only 13 cm and its annual clonal growth as less than 3 cm a year (Shirreffs

1985). Such distances are much too short to account for observed migration rates (Brunet and von Oheimb 1998a; Orczewska 2009). The importance of animal vectors for myrmecochore dispersal is also emphasized by studies from temperate deciduous forests showing that the majority of seeds or elaoiosomes on seeds fall prey to seed predators when dispersers are excluded or when rodents or carabids are abundant (Heithaus 1981; Ohara and Higashi 1987).

Third, a variety of arthropods and also gastropods feed on elaiosomes of myrmecochorous seeds (Mesler and Lu 1983; Gunther and Lanza 1989; Ohkawara et al. 1996; Türke et al. 2010), a fact that we observed in our video observations, too (Online Appendix A, table A2). Some arthropods other than ants transport seeds and might consequently disperse them. In North American forests, several predatory wasps (*Vespula* spp.) compete with ants for seeds and disperse greater numbers of *Trillium* spp. seeds (Jules 1996; Zettler et al. 2001; Bale et al. 2003). Several observations suggest that gastropods are relevant seed dispersers in forest biomes. A variety of studies in temperate forests in both Europe and North America have reported seed dispersal by gastropods. While gastropod feeding on fruits of strawberry, bilberry, huckleberry and other fleshy-fruited plants resulted in the 'accidental' consumption of tiny seeds (Müller(-Schneider) 1934; Müller-Schneider 1967; Gervais et al. 1998), slugs deliberately swallowed entire seeds of wood anemone and wild ginger, too (Türke et al. 2010). Moreover, seeds of wood anemone, *A. ranunculoides* L., *Carex* sp. and *Allium ursinum* were observed in the feces of wild-caught red slugs (Türke et al. 2010; M. Türke, unpublished data). Eventually, seeds, including those of wood anemone, are capable of germinating after gut passage (Müller(-Schneider) 1934; Müller-Schneider 1967; Gervais et al. 1998; Türke et al. 2010).

Fourth, it is also noteworthy that average seed dispersal distances by red slugs can be considerable (4.4 m; Türke et al. 2010), being about five times that performed by ants (merely 0.87 m; data from the northern hemisphere, reviewed by Gómez and Espadaler 1998). Actually, the magnitude of seed dispersal by red slugs appears to be most compatible with the observed spread and abundance of myrmecochores in European deciduous forests (Brunet and von Oheimb 1998a, 1998b; Orczewska 2009). Moreover, in our study, the abundance of myrmecochores was positively correlated with the abundance of terrestrial gastropods (fig 2), but not of ants (fig 1). This further suggests that gastropods may disperse myrmecochorous seeds, although not all gastropods caught by pitfall traps may act as seed dispersers. However, gastropod species other than red slugs might be effective dispersers, too.

Fifth, in our seed removal experiment we exposed seeds of wood anemone and wild ginger in forests of various management types in a large-scale approach to find general patterns of seed removal by different animal taxa in Central European beech forests. We

found great differences in removal rates between different animal exclusion treatments. The influence of insects and rodents to seed removal was low and most seed removal could be attributed to gastropods (fig 3). Because rodents are prominent post-dispersal seed predators in temperate regions (Hulme 1998), their abundance may have been low during our study due to the early-season in which experiments were conducted (Hulme and Kollmann 2005). In our experiment, there were slight differences in the amount of seeds being removed among regions, and hardly any influence of forest management on seed removal, suggesting that our results are representative of a wide variety of beech forests. Finally, it has to be emphasized that the actual fate of seeds that are removed from artificial depots as in our experiment is unknown and we can only speculate on whether they had been predated, dispersed or if a plant will establish from the seed at a new site.

Conclusion

Altogether, our results support the hypothesis that in mature shaded beech forests myrmecochores can be highly abundant despite the fact that ants are rare or absent because gastropods act as substitutes for ants as seed dispersers. Thus, we question the traditional view of ants as the only vector of “myrmecochorous” plant species and we encourage further study of the role of gastropods in the dispersal ecology of herbs also in other ecosystems as well. Surprisingly, gastropods might have been overlooked for decades in the dispersal ecology of myrmecochores. Given the increasing abundance of invasive gastropods (Chichester and Getz 1973; Martin 2000; Shea 2006, 2007), many of which are large enough to potentially disperse seeds, it will be interesting to study whether seed dispersal by gastropods may become even more pervasive globally.

Acknowledgements

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Appendix

Red slug (*Arion rufus*) consuming a seed of European wild ginger (*Asarum europaeum*). Photograph by Manfred Türke.

Online Appendix A: Extended methods and results

Table A1: Distribution of plots over different forest management types in the NE region (Schorfheide-Chorin), the Central region (Hainich-Dün) and the SW region (Schwäbische Alb). The plots where seeds had been exposed in the seed removal experiment are given in brackets.

	Region		
Management type	SW region	Central region	NE region
Beech extensively managed	5 (5)	13 (5)	7 (5)
Beech selection-cutting	0	13 (5)	0
Beech age-class forests			
Old timber	12 (5)	4 (3)	7 (5)
Old timber multilayered	6 (5)	0	0
Young timber	0	4(2)	0
Pole wood	10 (1)	4	0
Thicket with shelterwood	0	3	7 (5)
Thicket without shelterwood	5	5 (1)	0
Total number of plots	38 (16)	46 (16)	21 (15)

Note: In selection-cutting forests, indiviudal trees are harvested selectively and in age-class forests, all the trees are of the same age within distinct developmental stages.

<u>Evaluation of animal exclusion techniques using video recordings</u>

Materials and Methods

Plant material and video equipment

Seeds of wood anemone were collected in the Central region in May and of wild ginger in a forest in the vicinity of Jena in June 2010 and kept at -20° C until used in the experiment. A detailed description of the video cameras we used is given by Meyhöfer (2001). Videos were recorded and analyzed with the recording software eytron.®-VMS Basic Version 6.5.1620 (ABUS Security-Center GmbH & Co. KG). Videos were recorded non-stop over the whole period of the experiments taking two pictures per second. Videos were watched at 10 times speed and speed was reduced if observations of animals or activities were made.

Video recordings in the field

We installed seed depots with the four different treatments (1) unlimited access to all animals (control), (2) access to rodents and insects (gastropod exclusion), (3) access to gastropods and insects (rodent exclusion) and (4) access to insects (gastropod and rodent exclusion) at a site in the Hainich National Park, an unmanaged beech forest in the Central region. Seed depots were separated by four meters. The experiment lasted from 29.07.2010 to 06.08.2010. Seed depots were supplied with new seeds every day approximately at 2 pm. The number of seeds being removed during the previous day was recorded. Depots were supplied with either seeds of wood anemone or wild ginger on four days for each species. The plant species was changed every two days. Seed depots were not shifted but the positions of (1) and (2) and of (3) and (4) were exchanged after four days. Cameras were placed on a plastic box and adjusted with sticks found at the study site. To avoid great divergence in illumination between day and night, seed depots and cameras were shaded by dark blue plastic foil. The foil was not in contact with the forest floor at any position to avoid restricted entry of animals. Electricity to run a computer and the video equipment was obtained from a climatology measuring tower (Bioclimatology, University of Göttingen) located in 8 m distance to the closest seed depot. The computer was placed within the area of the measuring tower. We recorded every visit to the seed depots, identified the visitor as precisely as possible, and recorded whether the seeds were touched. However, it should be emphasized that video quality, mainly at night and especially in the cage treatments, was not always good and some small animals might have been missed. Not all borders of a seed depot were visible in the night and mainly smaller animals being deterred by the slug repellent might have been missed. The centre of the seed depot with the seeds was visible in all treatments with adequate quality. We compared visitation frequency separately for different animal taxa in a linear mixed-effects model (fit by the Laplace approximation for poisson errors) using the function ‘‘lmer’’ in the lme4 package. Treatments were treated as a fixed effect and the plant species and the date of an experiment were treated as the error. We conducted an F-test to test for a significant effect of treatments as:

```
K <- diag(length(fixef(model)))[-1,]
summary(glht(model, K = K), test = Chisqtest()).
```

Video recordings in the laboratory

We collected at least 100 workers along with pupae and larvae of five ant colonies in the field and brought them to a climate chamber. We refer to these groups as ant colonies in the following. Two colonies of the black-backed meadow ant (*Formica pratensis* Retzius) which belongs to the wood ant group were collected at the forest edge on a meadow in Jena,

Thuringia. One colony of the small black ant (*Lasius niger* L.) and two colonies of the common elbowed red ant (*Myrmica scabrinodis* Nyl) were collected at a forest edge in the Hainich-Dün region. We chose different species of three common ant genera for our experiment to test whether results can be generalized. Ant colonies were transferred with soil of their nest environment to plastic flower pots (11 × 13.5 cm; diameter × height). Each pot was placed in the right half of a plastic tub (53 × 30 × 11 cm; length × width × height) with walls being coated with Fluon (Fluon® GP1, Whitford Plastics Ltd., UK) to prevent escape of ants from the tub. In the left half of the tub we placed a basin of 21 cm diameter filled with planting soil (arena). In this arena we placed a seed depot outlined with slug repellent paste (as in the treatment with access to rodents and insects (gastropod exclusion)). Four bridges made of wire were fixed on stryrofoam stripes located next to the arena. Ants could enter seed depots either over the slug repellent or over the bridges. Cameras filmed the arena from 10.–12.08.2010. Constant conditions of light, humidity (75 %) and temperature (22 ºC) were maintained in the climate chambers throughout the experiment. Seeds of wild ginger were offered approximately at 5 pm on 10.08.2010. New depots were installed on 11.08.2010 with seeds of wood anemone approximately at 5 pm. An experiment lasted 24 hours. We recorded the first visitation of the depot by an ant worker and the first contact with a seed. We recorded every movement of seeds and described them by the following categories: (1) transport within depot, (2) seed was dropped into slug repellent paste, (3) seed was carried over the slug repellent paste and (4) seed was carried over a bridge. We further recorded the period during which an ant moved a seed. Time to carry a seed over a bridge was compared among ant species with ANOVA using the function ‘‘aov’’. Data was log-transformed.

Results (seed removal experiment)

Video recordings in the field

Different animals were observed to visit seeds, including beetles (Coleoptera: Carabidae, Staphylinidae), earwigs (Dermaptera), harvestmen (Opiliones), woodlice (Isopoda), slugs and snails (Gastropoda), rodents (Rodentia) and other smaller arthropods including flies (Diptera), springtails (Collembola) and individuals that could not be identified. We observed no ants in any seed depot. We suggest from the observations that among the arthropods mainly carabids are responsible for seed removal while the other taxa but also carabids are responsible for elaiosome damages (For an example video of a carabid crossing the slug repellent and removing a seed see Online Appendix C: Carabid removing seed.mpg). The

population of slugs had decreased drastically for unknown reasons at the study site in the National Park compared to previous months (M. Türke, personal observations). Only few red slugs (*Arion rufus*) were observed at the time of the experiment while earlier in the year several hundred individuals could be found per hectare. Thus, only few encounters of slugs and seeds were observed. In one case, a mature red slug entered a rodent exclusion treatment and swallowed all ten seeds (see video excerpt in Online Appendix B: Slug swallowing seeds.mpg). In another case, a red slug entered a depot protected by slug repellent and presumably swallowed six seeds. In total, gastropods were observed seven times visiting seeds. Rodent abundance, in comparison, appeared to be enhanced due to the facilities of the measuring tower. Individuals of the yellow-necked mouse (*Apodemus flavicollis*) and the bank vole (*Myodes glareolus*) were observed to consume seeds.

A total of 92 of 320 seeds (29%) were removed or destroyed, most of which were consumed by rodents. Gastropods swallowed 16 seeds (5%). Only few seeds were removed or obviously destroyed by arthropods. Carabids removed a total of 12 seeds (4%) from depots (1 in controls, 5 in treatments with access to gastropods and insects (rodent exclusion), 6 in treatments with access to insects (rodent and gastropod exclusion)) and destroyed two seeds within a depot with access to insects (rodent and gastropod exclusion). A total of 35 of 160 seeds of wild ginger (22%) showed feeding traces on elaiosomes (>50% of the elaiosome volume). We could not observe any feeding traces on seeds of wood anemone under the microscope although seeds were also visited by arthropods.

The observations clearly showed that the slug repellent deterred arthropods. Different arthropods were regularly observed to reach the seed depot, turning at the paste. Only in few cases arthropods crossed the paste. Smaller insects were often observed to enter and leave depots over the bridges, including earwigs, which entered 19 times and which we consider to be important consumers of elaiosomes. At least 46 other arthropods entered depots protected against gastropods. Slug repellent also reduced access of seed depots by carabids. Carabids entered 11 times but were deterred 28 times. However, as mentioned above, equal numbers of seeds were removed by carabids from depots protected by slug repellent and from depots that were not protected or only protected by a cage. There are some differences in the number of individuals of different taxa that visited seeds (not regarded are animals that entered seed depots but did not visit seeds) in the four treatments (table A2), which, however, can not only be described by the treatment itself but must also be related to variability of taxa abundance at the microsites (some taxa were not observed in certain seed depots at all).

In conclusion, the seed removal by arthropods was rather low and can mostly be attributed to carabid beetles. The use of slug repellent will probably underestimate the feeding damage inflicted on elaiosomes by arthropods but probably not the seed removal.

There will still be a fair proportion of elaiosome damage by arthropods that enter protected seed depots over the bridges or in the case of carabids over the slug repellent paste. Although the slug repellent deterred carabids quite often, they crossed the repellent several times, removing as many seeds as from depots without slug repellent.

Video recordings in the laboratory

Ants had no problems to enter seed depots over the bridges (see video of *F. pratensis* worker removing an *A. europaeum* seed in Online Appendix D: ant removing seed.mpg). The slug repellent deterred ants in the first hours of the experiment. After several hours the slug repellent started to dry out due to the activity of the fan in the climate chamber and as a consequence ants started to cross the repellent.

While ants of all colonies were interested in seeds of wild ginger, not a single seed of wood anemone was collected by ants. Seeds of wood anemone were not even recognized by the ants though many ants were observed within seed depots. Seeds had no feeding traces after 24 hours. In two cases, *M. scabrinodis* and *L. niger* used the depot as a refuse pile and covered seeds with dead ants and other material. Seeds were slightly moved onto the refuse piles. One colony of *F. pratensis* covered the whole depot and the seeds with soil without moving any seed. We conclude that the ants were not interested in seeds of wood anemone.

In contrast, we recorded 82 instances of seed transport by ants for wild ginger, 29% of which were only relocated within the depot and can thus be considered as elaiosome feeding (without seed dispersal). Elaiosome feeding was often observed also without seed movement mainly in *M. scabrinodis*. A total of 82% of the seeds offered were removed from seed depots and the majority (83%) was carried over the bridges while less seeds were transported over the paste (17%), mostly at a time when the paste had already dried out. Seeds dropped into the paste were picked up by other ants later and then removed from depots. All ant species carried seeds over the bridges. One colony of *M. scabrinodis*, however, mostly was feeding on elaiosomes in the first hours and then removed only a single seed over a bridge and four seeds over the paste. Ant species differed significantly in the time they took to transport seeds over bridges (ANOVA, $F_{2,31}$= 8.23, p=0.0014). *Formica pratensis* took 72±12s (mean ± SE) to transport a seed over a bridge, *L. niger* took 183±31s and *M. scabrinodis* took 103±24s.

We conclude that ant species of the most common genera are obviously able to carry seeds over the bridges. Thus, the gastropod exclusion treatment should not reduce seed removal by ants significantly in the field compared to controls.

Table A2: Number of seed visitations of animal taxa in depots of different animal exclusion treatments during the video recording experiment.

Animal taxon	Seed depot treatment (access of animal taxa)								GLMM
	All animals		Gastropods + insects		Rodents + insects		Insects		
	A. nem	A. eur	A. nem	A. eur	A. nem	A. eur	A. nem	A. eur	
Carabidae	4	5	3	20	0	1	6	3	**
Staphylinidae	0	3	0	8	2	0	0	1	*
Dermaptera	2	3	1	1	5	5	1	4	.
Other arthropods	3	27	1	8	3	5	2	0	**
Opiliones	2	4	1	1	0	0	0	0	ns
Isopoda	0	2	0	0	0	0	0	0	ns
Gastropoda	0	0	1	3	1	1	0	1	*
Rodentia	2	2	0	0	2	2	0	0	ns

NOTE: – A. nem = *Anemone nemorosa*; A. eur = *Asarum europaeum*. The results of a GLMM are given, testing for differences in seed visits of certain taxa between treatments (ns, $p>0.05$; ., $p<0.1$; *, $p<0.05$; **, $p<0.01$).

Table A3: Number of removed seeds (Rem) and seeds with elaiosome damage (Ela) in the seed removal experiment. More seeds were removed in the Central than in the NE region (GLMM, Tukey post-hoc test; z=3.57, p=0.0010) but there was no difference between the SW and the Central (z=-2.33, p=0.051) or the NE region (z=1.29, p=0.40). Management had no influence on seed removal (GLMM, Tukey post-hoc test; p>0.05), except for the NE region where more seeds were removed in the thickets with shelterwood than in old timber age-class forests (ACF) (z=3.52, p=0.0012) and unmanaged forests (z=3.42, p=0.0018).

				Access to seed depot							
				All animals		Rodents and insects		Gastropods and insects		Insects	
Plant species	Region	Management type	N plots	Rem	Ela	Rem	Ela	Rem	Ela	Rem	Ela
A. nemorosa	NE	Extensively managed forest	5	5±3	5±4	1±1	0±0	6±5	7±5	0±0	0±0
		Old timber ACF	5	3±2	1±1	0±0	0±0	3±2	1±1	0±0	1±1
		Thicket with shelterwood (ACF)	5	32±12	6±5	0±0	1±1	3±2	1±1	2±2	1±1
	Central	Extensively managed forest	5	49±11	14±4	16±5	0±0	46±11	7±3	19±10	3±2
		Old and young timber ACF	5	33±10	5±3	13±9	0±0	21±10	6±3	8±8	3±3
		Selection forest	5	52±7	9±5	1±1	0±0	43±14	2±1	5±3	0±0
		Thicket without shelterwood (ACF)	1	50±40	0±0	na	na	0±0	0±0	0±0	0±0
	SW	Extensively managed forest	5	49±11	14±5	1±1	0±0	26±12	2±1	1±1	0±0
		Old timber ACF	5	48±8	10±5	2±2	0±0	24±9	1±1	0±0	0±0
		Multilayered old timber ACF	5	58±13	5±2	1±1	1±1	25±13	4±2	0±0	0±0
		Pole wood (ACF)	1	10±10	5±5	5±5	0±0	10±10	0±0	0±0	0±0
A. europaeum	NE	Extensively managed forest	5	53±11	42±12	19±8	0±0	43±9	51±11	14±7	1±1
		Old timber ACF	5	60±9	22±7	8±5	0±0	56±13	6±2	10±4	0±0
		Thicket with shelterwood (ACF)	5	83±7	17±7	23±9	14±10	69±10	28±9	22±6	18±9
	Central	Extensively managed forest	5	68±13	22±10	15±10	0±0	45±12	50±11	1±1	0±0
		Old and young timber ACF	5	56±12	43±12	5±3	0±0	64±11	21±10	9±7	3±3
		Selection forest	5	78±9	22±9	39±16	11±9	43±12	29±11	18±12	0±0
		Thicket without shelterwood (ACF)	1	70±30	0±0	5±5	0±0	70±30	0±0	30±10	0±0
	SW	Extensively managed forest	5	57±12	16±10	3±2	7±5	50±11	30±11	8±4	9±8
		Old timber ACF	5	70±10	24±9	5±3	10±8	33±11	38±12	5±3	8±8
		Multilayered old timber ACF	5	73±10	24±10	8±4	18±11	29±8	35±11	12±10	8±8
		Pole wood (ACF)	1	95±5	5±5	15±5	0±0	45±15	55±15	0±0	0±0
				Mean±SE (%)							

Online video enhancements (videos are available upon request)

Online Appendix B: Slug swallowing seeds.mpg

Mature red slug (*A. rufus*) swallows wild ginger seeds (*A. europaeum*) after it has entered a seed depot with restricted access (rodent exclusion) in an unmanaged beech forest.

Online Appendix C: Carabid removing seed.mpg

Carabid beetle enters depot with restricted access (gastropod and rodent exclusion) over the slug repellent paste, picks up a seed of wild ginger (*A. europaeum*) and leaves the depot with the seed. Video was recorded in an unmanaged beech forest.

Online Appendix D: Ant removing seed.mpg

Worker of the black-backed meadow ant (*Formica pratensis*) carries a seed of wild ginger (*A. europaeum*) over an ant-bridge, leaving a seed depot with restricted access (gastropod exclusion) in the laboratory.

CHAPTER SIX

General Discussion

My studies were intended to assess the potential of gastropods to act as seed or elaiosome predators or dispersers of ant-dispersed plants (myrmecochores), mainly within European beech forests. Sketching the results of my research, everything is pointing to gastropods as significant seed dispersers of myrmecochores (question 1 in the general introduction). Individuals, however, may disadvantage plants if they consume elaiosomes instead of swallowing and dispersing seeds, as this might prevent dispersal by other gastropods or ants. On the other hand, seeds were never destroyed by gastropods in the feeding process.
The gastropod species tested differed in their seed feeding behavior (question 2). *Arion* species were feeding readily on all seed types offered and bear the potential to disperse them. Juvenile or small individuals, however, swallowed small seeds only and were devouring elaiosomes of large seeds. The two other gastropod species, *L. cinereo-nige*r and *C. hortensis*, were rather reluctant seed feeders, but at least certain plant species, represented by seeds of *M. perennis* in my studies (chapter 3), might suffer severe elaiosome predation. A few seeds were swallowed by these species, too.

Gastropods were feeding on seeds of all seven myrmecochorous plant species offered (question 3), but there were differences in the attractiveness of seeds to gastropods. Swallowing of seeds of *A. europeaum*, for instance, was disproportionately high in contrast to other plant species and deviated from the negative regression line of the relationship of diaspore size and the seed swallowing frequency. The seeds of *M. perennis* suffered most elaiosome predation, but only few seeds were swallowed.

Plants might benefit from gastropodochory for several reasons (question 4). First, dispersal distances by red slugs might even exceed those by ants (chapter 2). Second, gastropods might disperse seeds in habitats where ants are rare as is the case in shaded beech forests (chapters 2 & 5). Third, attractiveness of seeds defecated by slugs was decreased for rodents (chapter 2) and seeds which slugs had fed on (elaiosome feeding and swallowing of seeds and defecation) were less likely to be removed by animals in the field (chapter 3). This highlights the potential of slug-consumed seeds to escape predation. Other

possible reasons how gastropodochory might be of selective advantage to plants are discussed below.

The differential seed feeding behavior of red slugs showed to be dependent on body mass (question 5). Larger slugs swallowed more seeds and also consumed a higher proportion of fruit skin by swallowing seeds of the total fruit skin consumed than smaller individuals. Other reasons that might influence the seed feeding behavior are discussed below.

Our assumption that myrmecochores are abundant in beech forests, while ants are rare at the same sites was confirmed (question 6). Interestingly, gastropod abundance was positively correlated with the presence of myrmecochores.

With the knowledge we gained in the seed feeding experiments, the finding that gastropods removed most of the experimentally exposed seeds in beech forests, suggests that they bear the potential to substitute ants as seed dispersers (question 7).

In the following I will discuss my results in the context of concrete topics and will give perspectives on prospective research on gastropodochory in myrmecochores.

General or Occasional Mutualism?

Gastropods may play a crucial role in the seed ecology of plant species in certain habitats either as dispersers and / or elaiosome predators. However, the main question is whether this relationship can be generalized where both, myrmecochores and gastropods, coexist. Terrestrial gastropods are a very diverse group with about 35,000 species worldwide, living in a variety of habitats including grassland, agricultural landscapes and forests in many different geographical regions (Barker 2001; Bogon 1990) where also myrmecochores, probably more than 11,000 species worldwide, can be found (Beattie 1985; Lengyel et al. 2010; Sernander 1906; Ulbrich 1919).

Indeed, interactions of gastropods and seeds of myrmecochores were observed in different regions and habitats. In second-growth redwood forests in California, USA, (Mesler and Lu 1983) and in beech-maple woods in New York, USA, (Gunther and Lanza 1989) slugs were attracted to *Trillium* seeds. We observed removal of *A. nemorosa* and *A. europaeum* seeds by gastropods from experimental depots in South-western, Central and North-eastern Germany with a maximum distance of more than 600 km between sites (chapters 2 & 5). We also found seeds of different species in the feces of wild-caught individuals of the red slug (*A. rufus*) in the Hainich region in central Germany (chapter 2). Laboratory feeding trials pointed towards a rather general interest of certain gastropod species for myrmecochorous seeds with the ability to disperse these seeds (chapter 3). A few more observations were made on gastropod feeding and removal of myrmecochorous

seeds. Gastropods obviously visited and probably removed seeds of wood barley *(Hordelymus europaeus*), spring vetchling (*Lathyrus vernus*), bear's garlic (*Allium ursinum*) and oxlip (*Primula elatior*) from experimental depots in the Hainich region, where most of our experiments were conducted, too (E. Vockenhuber, pers. comm.). In addition, large Arionid slugs were collected in fragmented mixed beech forests in Northern Spain (Peña Mayor, in the province of Asturias) and ten ripe seeds each of primrose (*Primula vulgaris*) and green hellebore (*Helleborus viridis*), growing in the same forests, were offered to ten slug individuals in the laboratory along with lettuce as additional food (A. Valdés Rapado, pers. comm.). Slugs swallowed 75% of *P. vulgaris* seeds but neither consumed elaiosomes nor swallowed seeds of *H. viridis*. Furthermore, slugs were observed to feed on capsules of common snowdrops (*Galanthus nivalis*) and European wild ginger (Asarum europaeum), one of my main study species, in a riparian forest in the vicinity of Vienna, Austria (V. Mayer, pers. comm.). A similar finding was reported from deciduous forests north of Cantley, Quebec, Canada, where non-native slugs, the Orange-banded Arion (*Arion fasciatus*), were feeding on capsules of Canada wild ginger (*Asarum canadense*) (Muir 1997). At times when seeds were still immature, slugs consumed the flower tissue only and the seeds exposed beneath the slug holes turned black. When capsules contained ripe seeds, slugs were feeding on the capsule tissue and on the elaiosomes, but did not harm seeds. Seeds were sometimes scattered beneath the plants. However, this small slug species, which reaches only 4 to 5 cm body length (Bogon 1990), was obviously not capable of swallowing the large seeds of *A. canadense*.

Most of these observations, however, are rather anecdotal or study areas were restricted. Large-scale studies similar to our seed removal experiment in German beech forests could help to quantify the importance of gastropods for the seed ecology of myrmecochores in these habitats. Clear evidence for the generality of gastropodochory in myrmecochores can only be supported by further studies which have to be conducted in various habitats and among different study species. Seed removal experiments in the field, however, are laborious, seed removing taxa are often hard to distinguish and in most cases only a restricted number of study species can be tested. The use of animal exclusion techniques to assess the contribution of different taxa to seed removal is frequently used (we also used this method). However, there is no information but only assumptions on the animals involved in seed removal. I strongly recommend the use of non-stop video observation of seed depots to identify seed predators or dispersers and to assess their contributions, as I did (chapter 5). Seed feeding experiments under controlled conditions could highlight the potential of seed dispersal by gastropods more quickly than field experiments, if a number of gastropod species or individuals feed on and swallow seeds of a variety of plant species. Nevertheless, observations should be confirmed in the field, as

questions such as whether gastropods find the seeds in their natural habitat cannot be answered in laboratory experiments.

Interactions of Gastropods, Ants and Seeds

Coexisting gastropods and ants will presumably compete for seeds and both taxa will contribute to seed dispersal, as it was shown for yellow jackets (*Vespula* spp.) and ants competing for *Trillium* seeds in North American forests (Bale et al. 2003; Jules 1996; Zettler et al. 2001) and for Carabid beetles and ants competing for the seeds of *Erythronium japonicum* Decne. in deciduous mesic forests of Hokkaido in northern Japan (Ohkawara et al. 1996). I observed ground beetles removing and potentially dispersing seeds, too, during the video observations in an unmanaged beech forest (see the Appendix in chapter 5), where, however, no ants were observed, and in a seed feeding experiment with Carabids in the laboratory (M. Türke, unpublished data, 2010).
In addition, there is the potential for secondary dispersal of slug-defecated seeds by ants. Seeds of *A. europaeum* defecated by red slugs (*A. rufus*) exposed in a dry grassland in Jena, Germany, were collected by red ants (*Myrmica* spec.), though they appeared to be less attractive to ants than control seeds (M. Türke, unpublished data, 2007). The same pattern was found for slug-defecated seeds of great celadine (*Chelidonium majus* L.) and *Myrmica ruginodis* in the laboratory (Tremer 2010). Interestingly, if seeds were washed and cleaned from slug feces, there was no difference in removal rates to control seeds (Tremer 2010). Thus, some gastropod-dispersed seeds might further be dispersed by ants. However, as seeds in the above mentioned studies were picked out of feces (and either not cleaned or cleaned afterwards), it has still to be tested whether ants find seeds in slug feces and if they are able to unhinge them.

Seed dispersal mutualisms involving ants were shown to be susceptible to invasions of alien ant species with devastating effects on plant communities (Christian 2001; Traveset and Richardson 2006). As gastropod invasions have recently become a problem in several regions such as Central and Northern Europe (Fischer and Reischütz 1998; Kappes 2006; Kappes et al. 2009), North America (Chichester and Getz 1973; Martin 2000) and Australia (Shea 2006; Shea 2007), non-native gastropods might already have had an impact on established seed dispersal networks of ants and myrmecochores unperceived. Invasive gastropod species may also interfere with yet unknown seed dispersal mutualisms of native gastropods and myrmecochores. Therefore, I conducted seed feeding experiments with the invasive Spanish slug (*A. lusitanicus*) and compared the results to those of native red slugs (*A. rufus*) which are recently being displaced in open habitats in Central and Northern Europe by the non-native slug *A. lusitanicus* (H. Reise, pers. comm.; Fischer and Reischütz 1998;

Kappes 2006; Kappes et al. 2009). Seed feeding behavior, however, was very similar in both species (chapter 3), while large seeded species might probably be disadvantaged most. And it has to be considered that both species will presumably differ in other aspects of their ecology, which might impact on their seed dispersal potential.

Plant Benefits

Gastropod seed dispersal must not necessarily be of benefit to the plants. Most important is that defecated seeds are viable and germinable, which, however, has been demonstrated by my germination experiment (chapter 2) and in several other studies (Gervais et al. 1998; Honek et al. 2009; Kollmann and Bassin 2001; Müller-Schneider 1967; Müller(-Schneider) 1934). Then it is necessary to assess the quality of the benefit. Regarding myrmecochores, five hypotheses on the evolution of myrmecochory have been discussed and tested in individual studies on ant-myrmecochore interactions (Beattie 1985; Giladi 2006). Until now, only assumptions on the mechanisms in gastropod-plant interactions exist and further studies are needed to test the following hypotheses:

(1) The dispersal-for-distance hypothesis. It means that dispersal distance is a selective advantage if it is inversely related to seedling mortality. No study has yet tried to assess whether gastropodochory promotes a reduction in seedling mortality. However, seed dispersal distances by red slugs (*A. rufus*) at the median defecation time of *A. europaeum* seeds could range from 0 to 14 m (mean 4.4 m) (chapter 2) and are similar to or even greater than the distances to which seeds are transported by ants (Gomez and Espadaler 1998). Thus, at least certain gastropods may provide the same benefits in matters of the dispersal-for-distance as ants do.

(2) The predator-avoidance of dispersed seeds. This was supported for slug-defecated seeds in my laboratory experiment where rodent seed predators preferred control seeds to slug-consumed seeds (chapter 2) and in a field seed removal experiment (chapter 3). However, further field seed removal experiments ideally based on video monitoring are needed to confirm these assumptions. Moreover, the selective advantage of predator-avoidance has to be assessed for certain plant species. In my seed removal experiments with *A. nemorosa* and *A. europaeum*, the contribution of rodents to seed removal was low (chapters 2 and 5), but higher later in the year (the following year) at the time of the video observation experiment, which, however, might partly be due to an increased rodent abundance at the study site (Appendix in chapter 5). Finally, seeds might even be less attractive to seed predators, if they are not picked out but remain within slug feces.

(3) The competition-avoidance hypothesis suggests that dispersed seeds reach microsites with reduced interspecific competition. This thesis has not been tested for

gastropod-dispersed seeds. But inhumation of seeds is very important for many plant species, facilitating an advantage in seedling establishment compared to other plant species and this service could also be rendered by gastropods (Beattie and Culver 1982).

(4) The nutrient hypothesis suggests that seeds reach nutrient-rich sites (normally within an ant nest) when they are dispersed by ants. There is no evidence that gastropods deposit seeds at nutrient-rich sites. However, seeds are defecated by gastropods in batches of feces of partly digested plant and other material, which might provide substrate, nutrients or moisture for germination and seedling establishment. Mucus and gastropod fecal material provide 'resource hotspots' for microbes and the increased microbial biomass contributes to a high carbon and nutrient turnover in the surrounding areas (Barker 2001). In my study, the germination rate of slug-defecated and control seeds of *A. nemorosa* did not differ, but seeds had been picked out of the feces before they were planted in flower pots (chapter 2). Gastropod digestion, however, can accelerate the germination of seeds in some plant species (Gervais et al. 1998; Müller(-Schneider) 1934).

(5) The fire-avoidance of dispersed seeds. This was mainly supported for ant seed dispersal in fire-climax communities, where gastropods can be found as well (Barker 2001), but has not been tested according to gastropodochory, yet.

Gastropod Benefits

Seeds defecated by gastropods are generally viable and consequently might not suffer much from digestion (Gervais et al. 1998; Honek et al. 2009; Kollmann and Bassin 2001; Müller-Schneider 1967; Müller(-Schneider) 1934; Türke et al. 2010). The seeds of myrmecochores bear a nutrient-rich appendage, the elaiosome, which contains fatty acids, amino acids, carbohydrates and vitamins (Fischer et al. 2008; Mayer et al. 2005) and which is consumed by ants, other arthropods (Ohara and Higashi 1987; Ohkawara et al. 1996; Sernander 1906) and also by gastropods (chapters 2 and 3) (Mesler and Lu 1983). Often great parts of the elaiosome remained undamaged and were still attached to the seed after defecation by gastropods (chapters 2 and 3). To find out which nutrients are solved during slug digestion, the chemical content of seeds and elaiosomes before and after digestion could be measured. In this way, information on the efficiency of gastropod digestion of seeds and elaiosomes could be assessed and conclusions on the significance of seeds in the diet of gastropods could be given. Furthermore, it will probably answer why gastropods as observed swallowed seeds of non-myrmecochores without any attractant for animal consumers (Frömmig 1950; Honek et al. 2009; Kollmann and Bassin 2001). Yet, only analyses of the amino and fatty acid contents of slug-digested seeds and elaiosomes of *Asarum europaeum* and *Chelidonium majus* have been made (Reifenrath et al. 2010; Tremer 2010). Defecated seeds

and elaiosomes of *A. europaeum* had a similar content of amino acids and a similar relative composition of soluble amino acids and fatty acids as controls (quantities of fatty acids were not analyzed) (Reifenrath et al. 2010). Only the relative composition of protein-bond amino acids differed: the contents of arginine and histidine were reduced, the contents of threonine increased in defecated elaiosomes and lysine contents were reduced, but thyrosine increased in defecated seeds (Reifenrath et al. 2010). Concerning the contents of soluble N-containing substances, citrulline was reduced and ammonium increased in defecated elaiosomes. Results with *C. majus* indicated little difference in the total amino acid content of defecated and control seeds (Tremer 2010). However, the results of the analyses are somehow difficult to interpret. And, it appears that there are some changes occurring in the composition of amino acids and N-containing substances during gut-passage (Tremer 2010). Actually, utilization of nutrients (amino acids) from seeds and elaiosomes by slug digestion seems rather ineffective. Future work will probably have to include the analysis of fatty acid quantities, sugars and vitamins, too.

The Physiology of Gastropod Seed Feeding Behavior

In myrmecochores, gastropods may either swallow a seed entirely, consequently leading to seed dispersal or consume the elaiosome only, preventing dispersal (chapters 2, 3 and 4). Thus, it is necessary to figure out which factors influence this decision as the effects are either beneficial or disadvantageous for the plant. In my studies, the number of seeds consumed, the proportion of seeds swallowed, and the proportion of elaiosomes consumed, was very variable within gastropod species (chapters 2, 3 and 4). Some individuals, for instance, swallowed all seeds offered to them, while others only swallowed a few or none but consumed the elaiosomes of some seeds. In *A. rufus* this decision appears to be related to size restriction: in contrast to mature individuals, juvenile individuals swallowed no large seeds (chapters 2 and 3) and there was a negative correlation of slug body mass with the number of seeds swallowed (chapter 4). However, there must have been other decisive factors which induce mature slug individuals to swallow seeds. Plant species, for instance, differed in the proportion of seeds that were consumed and swallowed (chapter 3). While mature individuals of *A. rufus* swallowed 77 % of the *A. europaeum* seeds offered, for instance, only 15 % of *A. nemorosa* seeds were swallowed (chapters 2 and 3). A variety of other factors might possibly influence the seed feeding behavior of slugs, including abiotic factors such as temperature or humidity and biotic factors such as hunger or crowding/ competition of conspecifics, on which activity and behavior are generally dependent (Barker 2001). Conditions in my seed feeding experiments were kept constant and therefore no speculations on the influence of these factors can be made.

Dispersal of Non-Myrmecochores by Gastropods

Gastropods have been described as consuming and dispersing seeds of plants adapted to sundry dispersal vectors. Concerning the dispersal of fleshy-fruited plants (Gervais et al. 1998; Müller-Schneider 1967; Müller(-Schneider) 1934), it is obvious that vertebrates such as larger mammals or birds will achieve greater dispersal distances than gastropods. Nevertheless, seeds dispersed by gastropods might be of benefit for other reasons such as predator-avoidance or inhumation of seeds. However, this still has to be investigated in further studies. Additionally, the contribution of gastropods to the seed removal of fleshy-fruited plants has first to be quantified in different habitat types before is to be considered insignificant. Especially where larger vertebrate seed dispersers have become rare, slugs could still provide short distance dispersal and facilitate plant migrations.

At the moment it is inscrutable why gastropods, as has been observed, consume seeds without any structures for the attraction of animals, such as the seeds of anemochores (wind-dispersed plants), especially, as seeds seem not to have suffered much from digestion as they are defecated germinable (Honek et al. 2009; Kollmann and Bassin 2001). Feeding trials testing a variety of seeds with adaptation to abiotic dispersal vectors or autochory and several gastropod species, combined with germination experiments of gastropod-defecated seeds have to precede any further speculations in this field.

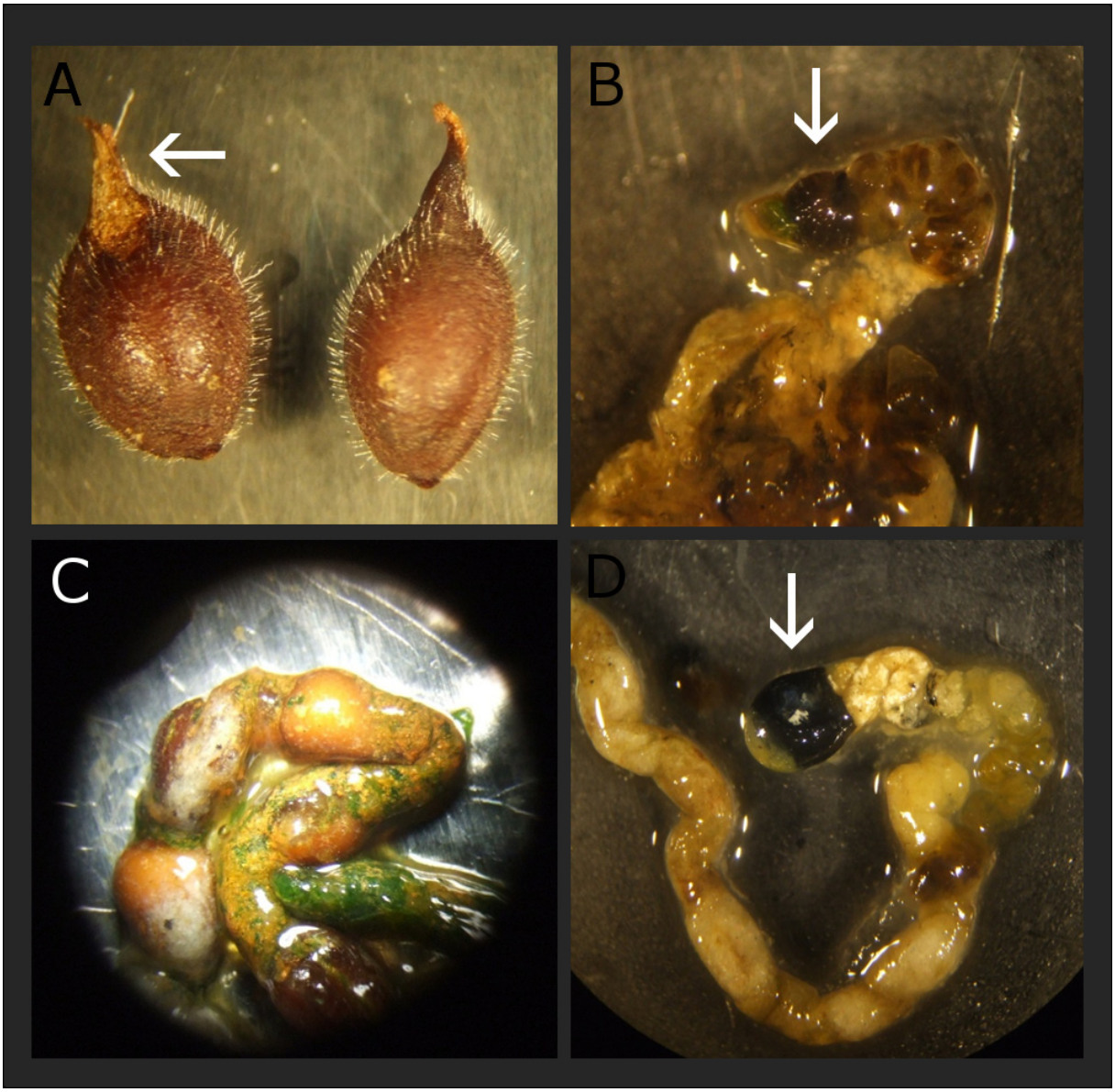
Conclusion

Gastropodochory has rarely been studied and has been discovered in myrmecochores only in recent times. In myrmecochores, gastropods may substitute ants as seed dispersers where ants are rare or absent. On the other hand, gastropods might interfere with ants as seed dispersers or elaiosome predators – especially if we think of invasive gastropod species. Future research will have to show if gastropods are important for plant migrations and distributions. Our knowledge on this new-found seed dispersal mutualism is still at its very beginning and will need intensive research. But as I could demonstrate, gastropods are obviously feeding and dispersing seeds, and therefore I strongly recommend accounting for gastropod seed feeding in studies dealing with the seed ecology of myrmecochores, but also of fleshy-fruited and other plants.

Das Buchenwald-Paradoxon

“Wenn ein Buchenbestand auf abgeholztem Boden aufwächst, unterdrückt er, je dichter er wird, den grössten Teil der lichtliebenden Kräuter und Gräser, die auf dem Schlage und in dem jungen Walde hervorgesprossen sind. Unter ihnen finden sich sehr spärlich Myrmekochoren, aber sehr merkwürdig ist es zu sehen, wie diese einwandern und sich immer stärker vermehren, je mehr Boden die Natur und die Beschattung erhält, die den fertiggebildeten Buschwald auszeichnen.“ Sernander (1906; p. 379)

“Die mit Abstand ameisenärmsten der untersuchten Habitate waren kronendichte Buchenalthölzer (…)“ Seifert (1986, p. 63)



Achene of (A) *Anemone nemorosa* with slug-consumed fruit skin at the persistent style (left) and untreated achene (right) and seeds of (B) *Lathrea squamaria*, (C) *Asarum europaeum* (5 seeds) and (D) *Allium ursinum* in the feces of red slugs (*Arion rufus*). Photographs by M. Türke, Jena, 2007.

ABSTRACT

The seed dispersal mode and ability of plants impact on plant migration, distribution and species richness and is of actual interest in restoration ecology, invasion biology and the response of vegetation types to global change. Several thousand plant species worldwide are adapted to seed dispersal by ants (myrmecochory) and they are a dominant part of many plant communities, as is the case in European beech forests. Ants, however, are often rare in beech forests due to unfavourable conditions. Thus, I suggested that there may be a discrepancy between the abundance of myrmecochorous herbs and their dispersers, ants. The consequence of this paradox might be that the majority of seeds fall prey to seed predators and myrmecochores migrate mostly by vegetative spread. Migration rates, however, regularly exceeded the distances achieved by vegetative growth. Therefore, I assessed the fate of myrmecochorous seeds in beech forests and suggested that animals other than ants might act as seed dispersers.

I compared ant, gastropod and myrmecochore abundance data from 105 differently managed beech forest plots distributed over three regions in Germany and I conducted seed removal experiments in 47 of these plots with seed depots which had restricted access for different animal taxa. I offered the diaspores of two forest myrmecochores, wood anemone (*Anemone nemorosa*) and European wild ginger (*Asarum europaeum*). As it turned out, gastropods contributed most to the seed removal and therefore I investigated the seed feeding behavior of gastropods in more detail. I offered seeds of seven forest myrmecochores to four gastropod species in the laboratory and I collected 309 red slugs (*Arion rufus*) in beech forests and searched for seeds in their feces. I concluded that gastropods can act as seed dispersers and, hence, tested their dispersal potential by comparing germination rates of slug-defecated and control seeds and by assessing the distances red slugs move in the field within the median gut-passage time of seeds in slugs. In addition, I tested whether slug-defecated seeds may escape predation and offered them along with control seeds to rodent seed predators in the laboratory and exposed them in the field.

The cover and species richness of myrmecochores in beech forests was negatively correlated with the abundance of ants but positively with the abundance of gastropods, supporting my assumption of a discrepancy in the presence of plants and their ant-

dispersers. Gastropods were most important for seed removal in my removal experiments, while rodents and insects including ants played only a minor role. Laboratory feeding experiments showed that gastropods consumed seeds of all plant species offered, but gastropod individuals differed in their seed feeding behaviors within and among species. Arionid slugs were readily feeding on seeds. Seeds consumed by gastropods were either swallowed as a whole or only elaiosomes, the nutrient-rich appendage on myrmecochorous seeds, were consumed. Swallowed seeds were defecated undamaged and germinated as well as control seeds in my germination experiment. I also discovered 19 seeds of different myrmecochores in the feces of wild-caught red slugs. Thus, gastropods may act as seed dispersers. The seed dispersal potential of gastropods, in particular, the proportion of seeds swallowed, was, however, dependent on the gastropod species, the plant species and the diaspore size and the size of gastropod individuals. Red slugs moved up to 14.6 m (mean 4.4 m) in 15 hours, the median gut passage time of seeds, and thus, might transport seeds even further than ants. Slug-defecated seeds were less attractive to rodents than control seeds, and in the field, far fewer slug-defecated seeds or seeds of which slugs had consumed the elaiosomes were removed by animals than control seeds, indicating the potential for gastropod-dispersed seeds to escape predation.

The results of my experiments indicate that myrmecochores might lack ants as their seed dispersers in beech forests, but they also suggest that ants could be substituted by gastropods. At least certain gastropod species, *A. rufus* in my studies, could be effective seed dispersers and provide significant dispersal distances and probably also protection of seeds against predators. Some gastropod species, however, will probably mainly act as elaiosome predators and might therefore disadvantage plants. In certain habitats, gastropods might compete with ants for seeds. This is of particular importance as gastropod invasions are occurring in many regions in the world, and might probably alter established seed dispersal mutualisms of native ants or gastropods and myrmecochores. Future work will have to test how important gastropods are for plant migration and distribution. As demonstrated in other studies, gastropods disperse seeds of non-myrmecochores, too, and should be considered as potential seed dispersers in prospective work on the seed ecology of herbs and shrubs wherever they can be found.

ZUSAMMENFASSUNG

Der Samenausbreitungsmechanismus und die Ausbreitungsfähigkeit von Pflanzen haben einen Einfluss auf Pflanzenmigrationen sowie ihre Verbreitung und ihren Artenreichtum. Samenausbreitung ist von gegenwärtigem Interesse in der Restaurationsökologie, der Invasionsbiologie und bezüglich der Reaktion von Vegetationstypen auf den globalen Wandel. Mehrere tausend Pflanzenarten weltweit sind an eine Samenausbreitung durch Ameisen angepasst (Myrmekochorie) und nehmen einen bedeutenden Anteil in vielen Pflanzengemeinschaften ein, so auch in europäischen Buchenwäldern. Ameisen sind in Buchenwäldern hingegen oftmals aufgrund ungünstiger Umweltbedingungen selten. Daher vermutete ich, dass es eine Diskrepanz zwischen dem Vorkommen von myrmekochoren Kräutern und ihren Verbreitern, den Ameisen, geben könnte. Die Konsequenz aus diesem Paradoxon könnte nun sein, dass ein Großteil der Samen Räubern zum Opfer fällt und Myrmekochoren sich hauptsächlich vegetativ ausbreiten. Migrationsraten überstiegen allerdings regelmäßig die Distanzen, die durch Wachstum zurückgelegt werden können. Deshalb untersuchte ich das Schicksal von myrmekochoren Samen in Buchenwäldern und vermutete, dass andere Tiere außer Ameisen die Samen verbreiten könnten.

Ich verglich die Abundanz von Ameisen, Schnecken und Myrmekochoren auf 105 unterschiedlich bewirtschafteten Untersuchungsflächen in Buchenwäldern, die über drei Regionen in Deutschland verteilt waren. Außerdem führte ich ein Samenentfernungsexperiment auf 47 dieser Flächen durch, in welchem ich Samen von zwei Waldmyrmekochoren anbot, dem Buschwindröschen (*Anemone nemorosa*) und der Haselwurz (*Asarum europaeum*). Verschiedene Samendepots waren nur für ausgewählte Tiergruppen zugänglich. Es stellte sich heraus, dass Schnecken am bedeutendsten für die Samenentfernung waren und deshalb untersuchte ich ihr Samenfraßverhalten genauer. Ich bot vier Schneckenarten die Samen von sieben Waldmyrmekochoren an und ich sammelte 309 Große Wegschnecken (*Arion rufus*) in Buchenwäldern und suchte in ihrem Kot nach ausgeschiedenen Samen. Aus den Ergebnissen dieser Untersuchungen schloss ich, dass Schnecken Samen verbreiten konnten und untersuchte deshalb ihr Samenausbreitungspotential, indem ich die Keimungsrate von Kontrollsamen und von Schnecken ausgeschiedenen Samen verglich. Ich ermittelte außerdem, wie lange Samen brauchten um den Darm von Großen Wegschnecken zu passieren und wie weit sich die Schnecken in dieser Zeit im Wald bewegten. Des Weiteren untersuchte ich, ob von

Schnecken ausgeschiedene Samen der Samenprädation eher entkämen und bot daher Nagern diese Samen zusammen mit Kontrollsamen zur Wahl an. In einem weiteren Samenentfernungsexperiment im Wald legte ich außerdem von Schnecken ausgeschiedene Samen zusammen mit Kontrollsamen aus.

Die Deckung und der Artenreichtum von Myrmekochoren in Buchenwäldern waren negativ mit der Abundanz von Ameisen, hingegen aber positiv mit der Abundanz von Schnecken korreliert. Schnecken waren wichtiger für die Samenentfernung als etwa Nager oder Insekten inklusive der Ameisen. Die Fraßexperimente im Labor zeigten, dass Schnecken die Samen von allen angebotenen Pflanzenarten fraßen, sich allerdings in ihrem Fraßverhalten innerhalb und zwischen den Arten unterschieden. Die Arioniden fraßen die Samen sehr bereitwillig. Die Samen wurden von den Schnecken entweder im Ganzen verschluckt, oder sie fraßen nur das Elaiosom ab, ein nährstoffreiches Anhängsel an myrmekochoren Samen. Verschluckte Samen wurden unbeschädigt ausgeschieden und keimten mit demselben Erfolg wie Kontrollsamen in meinem Keimungsexperiment. Des Weiteren fand ich 19 Samen von unterschiedlichen Myrmekochoren im Kot von den im Wald gesammelten Wegschnecken. Daher liegt es nahe anzunehmen, dass Schnecken Samen verbreiten können. Das Potential zur Samenausbreitung, also der Anteil Samen, der verschluckt wird, hing allerdings von der Schneckenart, der Pflanzenart und der Samengröße sowie von der Größe der Schnecke ab. Große Wegschnecken bewegten sich bis zu 14,6 m (Mittelwert 4,4 m) in 15 Stunden, der mittleren Darmpassagedauer von Samen in den Schnecken, und könnten die Samen daher sogar weiter ausbreiten als Ameisen. Nager bevorzugten Kontrollsamen anstelle der von Schnecken ausgeschiedenen Samen und im Wald wurden wesentlich weniger der von Schnecken ausgeschiedenen Samen von Tieren entfernt als von den Kontrollsamen. Dies weist darauf hin, dass von Schnecken ausgebreitete Samen der Samenprädation entgehen könnten.

Meine Ergebnisse zeigen, dass den Myrmekochoren im Buchenwald ihre Samenausbreiter, die Ameisen, fehlen könnten, aber auch, dass diese durch Schnecken ersetzt werden könnten. Zumindest bestimmte Schneckenarten – *A. rufus* in meiner Arbeit – könnten durchaus effektive Samenausbreiter sein, die signifikante Ausbreitungsdistanzen und unter Umständen auch einen Schutz vor Samenprädation gewährleisten. Manche Schnecken werden hingegen hauptsächlich Elaiosomen fressen und die Pflanzen eher benachteiligen. In bestimmten Habitaten werden Schnecken vermutlich mit Ameisen um die Samen konkurrieren. Dies ist von besonderer Bedeutung, wenn wir an die aktuell auftretenden Invasionen durch fremdländische Schneckenarten in vielen Regionen der Welt denken, die etablierte Samenausbreitungsmutualismen von einheimischen Ameisen oder Schnecken und Myrmekochoren beeinflussen könnten. Es wird die Aufgabe zukünftiger

Studien sein, die Bedeutung der Schnecken für die Migration und Verbreitung von Pflanzen zu bestimmen. In anderen Studien wurde außerdem gezeigt, dass Schnecken auch die Samen von nicht-myrmekochoren Pflanzen verbreiten, und daher sollten Schnecken als potentielle Samenverbreiter in kommenden Studien zur Samenökologie von Kräutern und Sträuchern unbedingt generell berücksichtigt werden.

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Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Dissertation mit dem Titel „Complicating a Complex Ecosystem Function: the Controversial Role of Gastropods in a Myrmecochorous Seed Dispersal Mutualism“ selbständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt habe.

Jena, April 2011 ……..

Manfred Türke

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