



Original article

Influence of elaiosome on postdispersal dynamics of an ant-dispersed plant

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ABSTRACT

Specialized myrmecochorous plants bear an elaiosome attached to the seed. Ants benefit from food provided by this appendage and plants get their seeds eventually dispersed. Aside from the benefits conferred by seed dispersal, we hypothesize that the influence of the elaiosome might extend to later life history stages such as emergence or survival, but few studies have examined rigorously such delayed effects on seed and seedling dynamics. This paper tests this hypothesis for the ant-dispersed herb *Helleborus foetidus*. Presence or lack of the elaiosome had some interesting delayed influences. Specifically, our results show that elaiosome removal not only enhanced seedling emergence, but also reduced cumulative long-term seed predation. The combination of these two effects, together with the inherent positive effect of the elaiosome on seed dispersal, highlights the ecological and evolutionary importance of myrmecochorous systems.

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1. Introduction

The mutualism of seed dispersal by ants, also known as myrmecochory, is a biotic dispersal mode widely distributed among herbs and woody plants. Its broad geographical and taxonomical distribution (Rico-Gray and Oliveira, 2007; Edwards et al., 2006), together with the fact it is one of the more important examples of directed dispersal, i.e. when seeds are mainly scattered in favourable conditions by animals (Howe and Smallwood, 1982; Hanzawa et al., 1988; Wenny, 2001; Wang and Smith, 2002), makes myrmecochory a key process in the demographical and evolutionary dynamics of a great number of plant species and communities worldwide.

Seeds of specialized myrmecochorous plants bear an attached elaiosome, a lipid-rich appendage attractive to ants. Ants transport diaspores (seed plus elaiosome) to their nests, where elaiosome is consumed, and eventually discard intact seeds, which are thus dispersed. Ants benefit from food provided by the elaiosome, which also facilitates seed manipulation, and plants get their seeds dispersed (Mayer et al., 2005; Giladi, 2006; Rico-Gray and Oliveira, 2007). Seeds so dispersed lose their elaiosomes and this removal may lead to consequences not directly linked to the dispersal event itself. The elaiosome is not a specific anatomical structure and may

have different tissular origins. The typical elaiosome usually develops from external seed integuments as a fleshy appendage in the micropyle area, but it may also originate from inner integuments (Lisci et al., 1996; Ciccarelli et al., 2005). Therefore, its removal by ants may entail different consequences for the final fate of the seeds. For example, in some species elaiosome removal may enhance germination by acting as a germination cue to break dormancy (Horvitz, 1981; Rockwood and Bloiss, 1986; Viegi et al., 2003; Ohkawara, 2005), while in others elaiosome presence may reduce germination (Viegi et al., 2003; Imbert, 2006) or have no effect (Rockwood and Bloiss, 1986; Viegi et al., 2003).

These consequences, apart from those exclusively linked to the dispersal event itself (density reduction, predator avoidance, and directed dispersal), may reinforce or weaken the ecological and evolutionary consequences of this mutualistic system. Therefore, it is necessary to widely investigate the consequences of elaiosome presence or removal by isolating them from those directly linked to the dispersal event, and examining elaiosome influence on seed and seedling postdispersal dynamics, including emergence and survival or even establishment, and direct reproductive success of new descendants.

Considering these probable consequences, three different scenarios are possible: first, positive effects of elaiosomes on dispersal are counterbalanced by negative elaiosome effects on posterior life history stages, i.e. by preventing or reducing the probability of seed germination and seedling emergence and survival (Viegi et al., 2003; Imbert, 2006); alternatively, it is possible that dispersal advantages conferred by the elaiosome are

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reinforced by positive effect of the elaiosome on later dynamics, i.e. by favouring germination, emergence and survival (Horvitz, 1981; Rockwood and Bloiss, 1986; Viegi et al., 2003; Ohkawara, 2005). The latter would greatly improve the advantages traditionally attributed to myrmecochory mutualism, which has been associated merely with the dispersal event. A last scenario would be that elaiosome has no effect apart from facilitating the scattering of seeds by ants.

Here, we assess the influence of elaiosome on postdispersal seed and seedling dynamics to test if advantages attributed to this dispersal device transcend the mere dispersal event. More specifically, we investigate if seeds that retain the elaiosome obtain better fitness results than seeds with the elaiosome removed. The following questions are addressed: (i) Do seeds with elaiosome differ from seeds without it in early postdispersal removal by rodents, in long-term postdispersal losses by rodents or fungi, in emergence, and in first year survival? (ii) Do such differences, if found, support the traditionally assumed advantages of myrmecochory?

2. Methods

2.1. Study species and sites

Helleborus foetidus L. (Ranunculaceae) is a myrmecochorous, rhizomatous perennial herb widely distributed across Western Europe and also present in north-western Africa (Werner and Ebel, 1994). Details of the floral biology of the species in the Iberian Peninsula, where it appears in the understorey of deciduous and mixed forests, in patchy scrub and ruderal habitats, can be found in Herrera et al. (2001, 2002). It flowers in winter (January–March), producing flowers with 2–3 carpels, each of which develops 10–15 elaiosome-bearing seeds. Carpels release diaspores in late June and July. Diaspores experience a dormancy of 18–21 months (Garrido et al., 2005), with most seeds germinating in the second year. Seedling emergence takes place from December to May. Although hellebore plants mainly grow by adding new ramets, their spatial spread and colonization of new patches are exclusively based on seed dispersal (Rey et al., 2006) and the species' demography is largely governed by seedling recruitment (Ramírez, 2006).

Main postdispersal hellebore seed removers, both predators and dispersers, are rodents and ants, respectively; and their influence on ecological and demographic dynamics of hellebore were studied in detail by Garrido et al. (2002) and Fedriani et al. (2004). Ant species (overall 36, principally species of the genera *Aphaenogaster*, *Camponotus*, and *Lasius*), their communities and their functional roles (mostly seed dispersers vs. very rare granivores) were assessed by Garrido et al. (2002) at the same three regions. The main small mammal seed predator was *Apodemus sylvaticus*, although some individual of *Mus domesticus* were also rarely reported at Caurel (Fedriani et al., 2004).

Experiments were carried out between June 1998 and May 2002 in four Iberian localities: 'Caurel' (Mostad, 1200 m.a.s.l., Sierra del Caurel, Lugo Province), 'Cazorla' (Aguaderillos, 1200 m.a.s.l., Sierra de Cazorla, Jaén Province), 'Mágina' (Puerto de La Mata, 1650 m.a.s.l., Sierra Mágina, Jaén Province), and 'Segura' (Collado de Góntar, 1650 m.a.s.l., Sierra de Segura, Jaén Province). The first was located in the northwest of the Iberian Peninsula and the three latter in the southeast. Localities were representative of the range of habitats most often occupied by hellebore (see Garrido et al. (2002), for further details on vegetation and localities). In each locality, seeds used for all experiments came from 15 local hellebore individuals.

Studies on emergence have traditionally started from seeds directly collected from mother plants, stored for some time in

different places and conditions, and finally sown. Conditions of this 'supposed' dispersal process may be dramatically different from those experienced by seeds in nature. In fact, recent studies on seed ecology show the importance of considering all of the processes experienced by seeds from their departure from the fruit to germination (Skarpaas and Stabbe, 2001). This requires keeping seeds in the places where they came from, under natural conditions, until experimental sowing takes place. Through this study we proceeded in this way, and thus seeds remained exposed under natural conditions until experimental sowings took place. Moreover, all sowings were placed far enough from hellebore shoots to prevent the presence of pre-existing seeds.

2.2. Short-term seed removal

Once diaspores are released by the plant, ants and mice find complete diaspores (i.e. seed plus elaiosome). Thus, this study of the seed removal at the short term was assessed by offering only complete diaspores with known traits (seed mass, elaiosome mass, and elaiosome to seed mass ratio).

This experiment was carried out in Caurel, Cazorla and Mágina in July 1998, just after the period when seeds are released. Two populations were selected in each locality, one in a patchy scrubland, and another in forest habitat (*Pinus* forest in Caurel and Mágina, and *Quercus* mixed forest in Mágina). In each population, fifteen experimental units were systematically distributed along 3 parallel lines, 4 m away from each other. Each experimental unit consisted of four different exclusion treatments: ants excluded, mice excluded, both excluded (experimental control), and neither excluded, following the exclusion methods and the experimental layout described in detail in Fedriani et al. (2004). Each experimental unit offered diaspores from a single, local mother plant. Diaspores from each mother plant used were characterized by measuring their seed mass (whole diaspore minus the elaiosome) and elaiosome mass (an absolute estimate of ant reward), and also by obtaining their elaiosome to seed mass ratio (reward relative to the effort that ants must invest in carrying a seed). Therefore, to analyze the effect of these characteristics on seed removal by ants and mice, we related the removal rates obtained from the exclusion experiment to the diaspore traits characterized for each mother (i.e. mean values per mother plant) plant in each experimental unit (see further details on seeds characterization in Garrido et al. (2002)).

2.3. Long-term seed fate

The long-term influence of the elaiosome on seed losses due to mice and fungi was assessed by exposing seeds with and without elaiosome to the action of these agents. Seeds were kept in natural conditions from June, when dispersal takes place, to the sowing period in December. During this time, seeds remained at the same sites where they were eventually sowed and placed into fiberglass mesh bags (mesh size 1 mm). Seeds were finally re-counted and classified as either 'predated by mice' (seeds completely or partially bitten by rodents), 'infested by fungi' (rotten seeds, or with any fungal signs), or 'healthy' seeds (completely intact seeds). Although this period does not cover the whole 18–21 months dormancy period, we considered it representative of the action of these agents, as detectability by mice and impact of fungal infestation are expected to decline with time, as seeds dry up and get deeper into the soil (Hulme, 1998).

2.4. Emergence and survival

Influence of the elaiosome on seedling emergence was explored by sowing seeds with and without elaiosome and subsequently

monitoring seedling emergence. This experiment was carried out between June 1999, when seeds were collected, and May 2001, when emerged seedlings were recorded. Once collected, seeds were kept in natural conditions from June to October, when sowing took place. During this period, seeds were kept inside fiberglass bags of 1 mm mesh-size (to prevent removal by ants), which in turn, were placed in metal-cages of 1 cm mesh-size (to prevent removal by mice). When sowing period arrived, fiberglass bags were removed, unlike the metal cages, which were kept until the end of experiment. Emerged seedlings were monitored monthly to determine their survival during their first year.

Experiments on long-term seed fate, emergence and survival were developed using a randomized blocks design without replication (Underwood, 1997, pp. 385–389), setting up ten blocks in each locality. As microhabitat influences emergence of hellebore seedlings by modifying water and light environmental conditions (Ramírez et al., 2006; Garrido et al., 2007), elaiosome effect was tested in different microhabitats. Therefore, in each block, elaiosome effect (2 levels: presence and absence) was completely crossed with microhabitat effect [3 levels: covered (microenvironment completely covered by shrubs), partially covered (with the same influence of cover than uncovered conditions), and uncovered (without any cover)]. Thus, 20 seeds with elaiosome and 20 seeds without elaiosome were located in each microhabitat of a block, which involved up to 120 seeds per block, 1200 per locality, and 3600 for all localities.

2.5. Data analysis

Short-term seed removal – Removal from each exclusion type was related to seed traits (seed and elaiosome mass and elaiosome to seed mass ratio) by means of two different regression models: a multiple one to test seed removal (number of seeds removed from Petri dishes) against seed and elaiosome mass (the two last variables were not significantly correlated: $r = 0.03$, $P = 0.844$) and a simple one testing seed removal against elaiosome to seed mass ratio. These analyses were only conducted with data from Mágina and Caurel and the locality factor was discarded, as previous analyses showed no differences between these two localities in the probability of seed removal (see Results: Long-term seed fate).

Long-term seed fate, emergence and survival – Dependent variables were the proportion of seeds consumed by mice or infested by fungi (in the case of long-term seed removal assessment), and emergence and survival probabilities. All of them were adjusted to a binomial distribution using logit link function. Analyses were conducted with GLIMMIX Macro of SAS/STAT software (SAS-Institute, 2003) which allows correct management of random factors in Generalized Linear Models.

3. Results

3.1. Short-term seed removal

Elaiosome mass did not affect the probability of seed removal in any of the exclusion types (Table 1), which indicates that neither mice nor ants discriminated among seeds depending on elaiosome mass. In contrast, seed mass did show a high influence on removal by ants, by mice and by ants + mice (none-excluded treatment combination). This influence showed quadratic relationships, although the latter was only marginally significant (Table 1). Removal by ants exhibited a disruptive selection pattern: it was higher with large or small seeds, and lower with medium-sized seeds (Fig. 1a). Inversely, removal by rodents followed a stabilizing selection pattern presenting the highest values with seeds of

Table 1

Logistic binomial regressions exploring the effects of the size of elaiosome (EM) and seed (SM) on removal by ants, mice, and ants + mice. Quadratic terms were also included to account for possible non-linear effects. Significant effects are shown in bold.

Removal agent	Ants		Mice		Ants + Mice	
	$F_{(1,30)}$	P	$F_{(1,30)}$	P	$F_{(1,30)}$	P
EM	1.32	0.259	0.93	0.343	1.71	0.201
SM	3.35	0.077	5.81	0.022	0.90	0.349
EM ²	1.18	0.286	0.89	0.353	1.00	0.327
SM ²	10.24	0.003	6.87	0.014	3.44	0.073
SM * EM	0.31	0.580	0.49	0.489	0.06	0.812

intermediate weight (Fig. 1b). Removal exerted by ants + mice was similar to removal by ants (Fig. 1c).

3.2. Long-term seed fate

In the long-term, 77.4% of the seeds were destroyed by mice, 3.8% were spoiled by fungi and 18.8% remained apparently healthy. The high seed predation by mice during this period probably makes this predatory event the most important seed drain in the demographical dynamics of this species.

Elaiosome, microhabitat and locality effects on long-term seed predation by mice were marginally significant (Table 2a) and interpretation of these effects may be done cautiously. Predation was slightly lower on seeds without elaiosome than on seeds with this structure (Fig. 2a). It was also lower under uncovered conditions than under the other microhabitats, and lower in Mágina than in the other localities (Fig. 2a). In addition, the elaiosome effect was consistent in space both locally (among microhabitats) and regionally (among localities), as there was no interaction effect. Finally, the locality × microhabitat interaction effect, although significant, will not be further examined since it falls out of the scope of this paper (see further information on locality and microhabitat effects for the same species in Garrido et al., 2005, 2007).

Seed losses by fungi, accounting for only 3.8% of total seeds, were negligible compared to the overwhelming seed losses by mice. Cazorla was not included in this analysis as its seeds did not show any signs of fungal infestation. None of the factors significantly explained variation in fungal infestation (Table 2b). Nevertheless, the lack of fungal infestation in Cazorla should imply a locality effect.

3.3. Emergence and survival

Elaiosome, microhabitat and locality affected emergence to some extent (Table 2c). Elaiosome presence reduced emergence probability in partially covered microhabitats ($\chi^2_1 = 7.78$, $P = 0.0053$), where it halved the emergence of the seeds (0.14 ± 0.029 vs. 0.28 ± 0.039) (Fig. 2b).

Microhabitat effect depended on locality (significant locality × microhabitat interaction, Table 2c), reflecting regional variation in how microhabitat influences emergence (Fig. 2c). There was also a simple effect of locality on emergence, which differed among localities. Remaining interactions were not statistically significant, showing a high geographical consistency in the effect of elaiosome on emergence (not significant elaiosome × locality interaction).

Elaiosome presence had no effect on seedling survival (Table 2d). The significant locality × microhabitat interaction indicates that microhabitat effect varied for each locality.

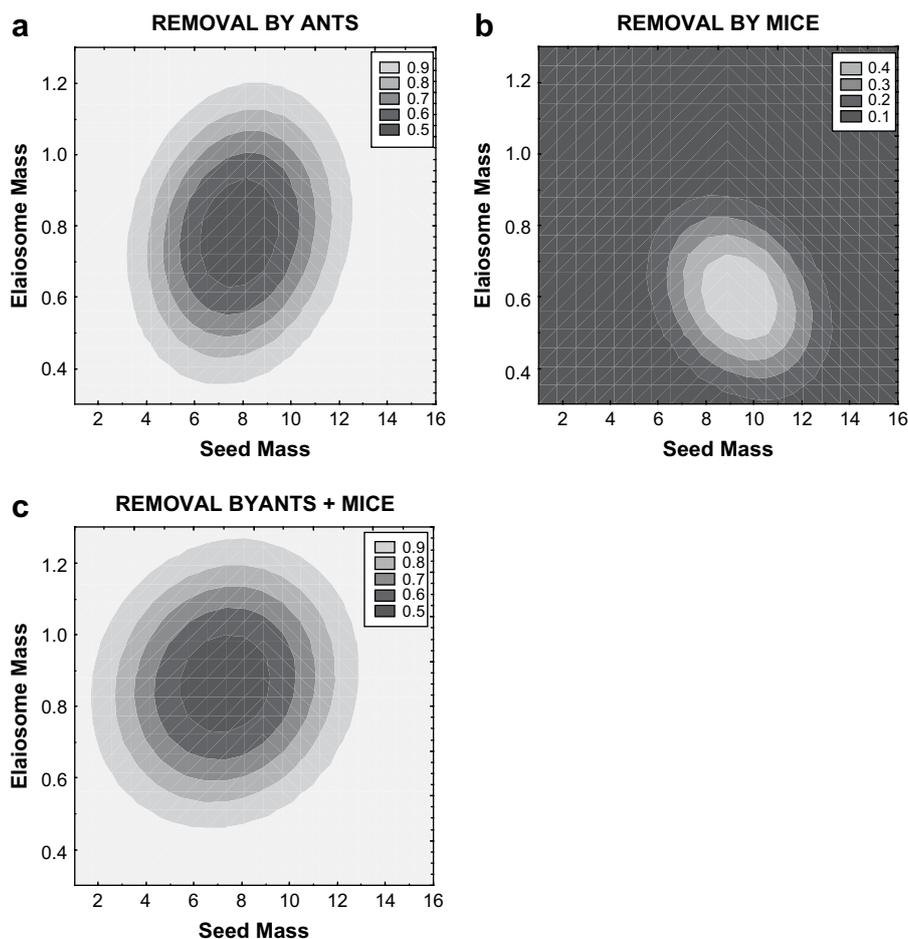


Fig. 1. Surface plots representing the probability of seed removal (Z-axis) by (a) ants, (b) mice, and (c) ants + mice, depending on elaiosome and seed masses (mg). Removal by ants, and by ants + mice, followed a disruptive selection pattern, whereas removal by mice followed a stabilizing selection pattern. In all cases only seed mass was able to influence on removal, as reflected in the model of Table 1.

4. Discussion

4.1. Effects of diaspore traits on ant and rodent short-term seed removal

Elaiosome size did not influence the pattern of short-term seed removal by either ants or rodents. The role of the elaiosome in hellebore dispersal has been further examined in other studies with this species (Garrido et al., 2002; Boulay et al., 2006, 2007; Alcántara et al., 2007; Manzaneda et al., 2007). Though the role of

elaiosome mass in other ant-dispersed species has been largely recognized (e.g., Edwards et al., 2006), overall, it seems that variation in its chemistry rather than in its mass is involved in determining inter-individual differences in seed removal rates in this species. In contrast to the negligible role of elaiosome mass, studies on hellebore dispersal have shown a significant effect of seed mass on ant removal (Garrido et al., 2002; Boulay et al., 2006, 2007; Alcántara et al., 2007; Manzaneda et al., 2007). Most frequently, ant assemblages seem to exert a disruptive selection on hellebore seed mass, a result also found here. A plausible explanation of this

Table 2
Generalized Mixed Linear Models exploring elaiosome, microhabitat and locality effects on: (a) seed predation by mice, (b) seed infestation by fungi, (c) seedling emergence, and (d) seedling survival at first year. Significant effects are shown in bold type and marginally significant effects in italics. (*) Non estimable variation for this factor. Except for fungal infestation, locality and microhabitat established differences across all phases. Elaiosome affected emergence differently in different microhabitats. Triple interaction was eliminated since it never was significant in any of the analyses.

Fixed factors	a) Predation by Mice				b) Fungal infestation				c) Emergence				d) Survival			
	ndf	ddf	F	P	ndf	ddf	F	P	ndf	ddf	F	P	ndf	ddf	F	P
Elaiosome	1	137	3.32	0.070	1	69	1.33	0.253	1	141	1.20	0.275	1	105.9	0.45	0.504
Microhabitat	2	138	2.68	0.072	2	69	2.32	0.105	2	141	0.13	0.875	2	113	2.25	0.110
Locality	2	25.8	2.99	0.067	1	69	1.42	0.236	2	30.7	5.73	0.007	2	31.22	12.4	0.000
Elaiosome × Locality	2	137	0.48	0.618	1	69	0.37	0.542	2	141	0.25	0.778	2	106.5	1.15	0.321
Elaiosome × Microhabitat	2	137	0.24	0.788	2	69	1.12	0.332	2	141	3.87	0.023	2	106.7	1.34	0.266
Locality × Microhabitat	4	138	4.49	0.001	2	69	1.03	0.362	4	141	3.66	0.007	4	113.8	3.93	0.005
Random factors			Z	P			Z	P			Z	P			Z	P
Block (Locality)			2.64	0.004			(*)				2.11	0.017			2.01	0.022

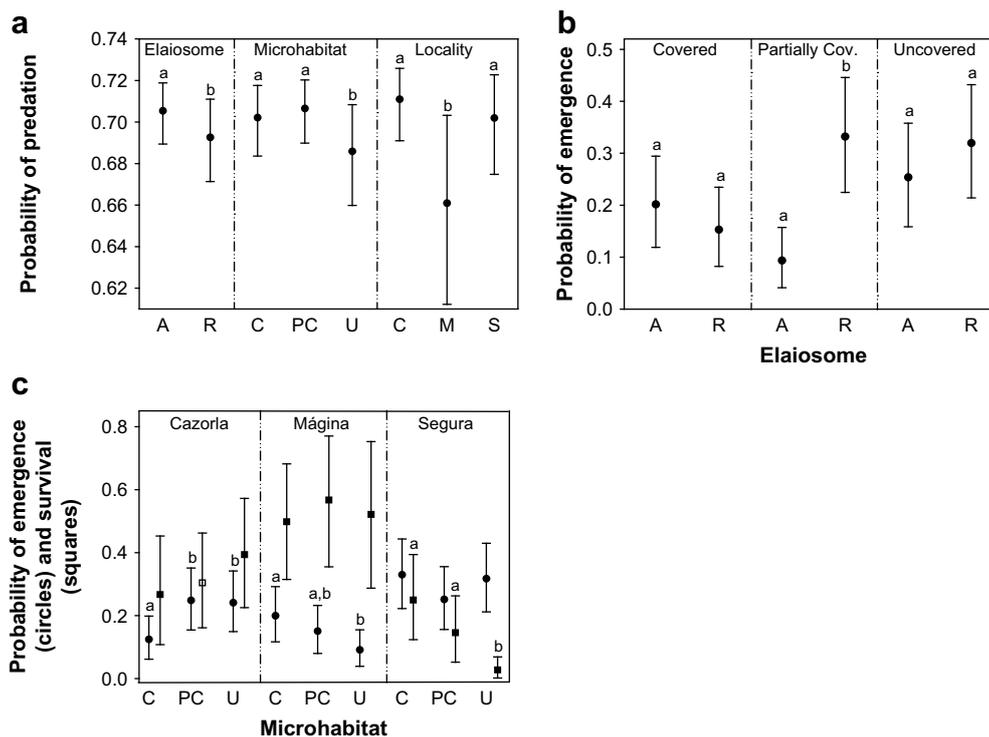


Fig. 2. (a) Differences in medium-term predation by mice due to presence of elaiosome (A: attached, R: removed), microhabitats (C: covered, PC: partially covered, and U: uncovered) and localities (C: Cazorla, M: Mágina, and S: Segura). Seeds without elaiosome, from Mágina, and at uncovered microhabitats tended to suffer lower predation. (b) Differences in emergence among microhabitats depending on the presence (A: attached) or absence of elaiosome (R: removed). Emergence of seeds without elaiosome doubled that of seeds with elaiosome in partially covered microhabitats. (c) Differences in seedling emergence and survival among microhabitats for each locality. High geographical variation in microhabitat influence determined locality-specific microenvironmental conditions. Therefore, microhabitats did not behave the same in different localities. Despite this, survival usually was lower in uncovered microhabitats. In a), b), and c) data are the model adjusted means with their 95% confidence intervals; different letters indicate significant differences, within each level, at $P < 0.05$, except in a), where differences were marginally significant, and distinct letters indicate differences at $P \leq 0.072$.

pattern is related to the ample ant disperser assemblage of this species, which involves both small (e.g., *Crematogaster*, *Leptothorax*, *Tetramorium*, *Lasius*, *Tapinoma*, and *Pheidole*) and large ants (*Formica* and *Camponotus*) (Garrido et al., 2002; Rey and Manzaneda, 2007). Large and small ants show very different capabilities to carry large diaspores (Garrido et al., 2002) and differently favour both extremes of the size distribution of the seeds in this species, generating disruptive selection (see also Alcántara et al., 2007).

Regarding predation by rodents, many studies have shown that bigger seeds offer more food to predators and are thus preferably consumed (Westoby et al., 1992; Herrera et al., 1994). Other studies contradict these results, specifically those dealing with hard-covered seeds (Willson and Whelan, 1990; Hulme, 1993; Westoby et al., 1996; Alcántara et al., 2000; but see Herrera et al., 1994). Hellebore seeds predation by rodents was not related to the elaiosome size, but it was related to seed size, with rodents preferably consuming medium-sized seeds. Thus, it can be suggested that small and large seeds escaped rodent predation more frequently than did medium-sized seeds. Since ants detect diaspores more quick than do rodents (Fedriani et al., 2004), it seems that rodents consumed what ants discarded (that is, medium-sized seeds). To summarize, high levels of dispersal against low rates of short-term predation, together with the influence of seed size on the general pattern of natural postdispersal removal, point to the existence of a disruptive selection pattern in the short term, favouring both small and large seeds.

Differential seed removal by rodents and ants has been previously documented in this species, and reflects the fact that ants were able to quickly remove most released seeds (Fedriani et al., 2004). Moreover, hellebore seeds are overall more quickly detected

by ant dispersers than by rodent predators. However, in some populations, rodents were able to interfere in the ant dispersal mutualism by preventing seeds from being dispersed by ants (Fedriani et al., 2004). Although in the present study we did not perform a rigorous phenotypic selection analysis on seed dispersal traits, it becomes apparent that elaiosome-bearing seeds, like other animal-dispersed seeds, are involved in selection conflicts between dispersal and survival-related traits (predation escape, emergence, survival and establishment success) (Alcántara and Rey, 2003; Martínez et al., 2007). This conflict was first apparent in seed removal because seed removal by ants and rodents (which determine opposing seed fates) showed non-linear opposing patterns of selection (disruptive vs. stabilizing). However, the final outcome of this opposing pattern on fitness needs to be further examined.

4.2. Long-term seed fate

Long-term seed consumption was twice as great as short-term seed predation (almost 70% of seeds escape from short-term predation). Thus, it can be stated that long-term seed predation may be the major source of seed loss in *H. foetidus*. Furthermore, during this period, elaiosome removal tended to reduce seed predation, which is of particular relevance in myrmecochorous systems.

Hellebore forms soil seed banks in which seeds can remain dormant at least 18–32 months (Garrido et al., 2005). Thus, seeds escaping from short-term seed predation, may eventually be destroyed by subsequent long-term predation or pathogen activity, exerted for example by mice and fungi, respectively (Herrera, 2002, and references therein). During six months we found very high

seed predation by mice and minimal fungal infestation (around 4%). After this time, seeds become deeply buried and we do not expect subsequent increased levels of predation by rodents (Hulme, 1994; Hulme and Borelli, 1999). Similarly, since prior to being examined, seeds had remained in the ground during the cool to warm humid seasons, we do not expect subsequent increased levels of fungal attack. Furthermore, routine examination of seed depots (data not shown) showed that long-term detection was not intensive, but sustained over time. Therefore, it may be assumed that long-term predation will be inversely proportional to the time that seeds remain buried, and that it will eventually decrease after seeds are too deeply buried.

These kinds of long-term postdispersal seed losses have been traditionally included in the overall postdispersal seed viability loss (Murdoch and Ellis, 2000, and references therein). Although they may represent a key process in population dynamics of species with prolonged dormancy periods, few studies have examined their causes and, consequently, much more effort is needed to clarify this crucial period.

Aside this quantitative relevance, which has an impact mainly in demographic terms, long-term seed predation of hellebore has an additional feature that highly increases its ecological and evolutionary significance: the elaiosome tends to increase long-term seed predation. In the context of ant–plant interactions this means that elaiosome removal by ants, an inherent fact in this dispersal mode, reduces the risk of long-term predation, probably the major seed drain in *H. foetidus*. Nevertheless, this result must be cautiously considered since the effect is not as strong as could have been expected. In short, elaiosome removal takes on special significance, since it presumably avoids a potentially strong predation, rising up plant fitness. This effect may be enhanced if, like we show in the next paragraph, removal of the elaiosome confers additional advantages.

4.3. Emergence and survival

Our results show large regional variation in emergence levels, which are lowest at Mágina. This broad regional variation suggests that conditions generated by each microhabitat are different at each locality. Microhabitat categories used here are too broad and perhaps represent locality-specific environments. In any case, it seems that conditions imposed by the uncovered microhabitat are the worst for emergence and determine to a large extent the high regional variation found in microhabitat effect. Determinants of the spatial variation (within and among localities) in seedling emergence in hellebore are out of the scope of this paper and have been investigated elsewhere (Garrido et al., 2005, 2007; Ramírez et al., 2006; Rey et al., 2006). Here we will focus on elaiosome-related effects on seedling emergence and survival.

For some species with elaiosome-bearing seeds the influence of this appendage on emergence has been proved (see references in Introduction). In some species, the elaiosome acts as a 'bridge' between the embryo and the external environment, thus favouring imbibition (Lisci et al., 1996). In other species, elaiosome removal frees an opening on the seed coat which favours water intake and the subsequent embryo imbibition (Horvitz, 1981). This seems to be the case in this species, as stated below.

Our work shows that the elaiosome has an important impact on seedling emergence. Specifically, elaiosome removal doubled the rate of emergence in partially covered conditions, although there was not such an effect under the extremes of cover conditions (completely covered or uncovered). It seems that in extreme environments such as open interspaces with very dry soils (typical in Mediterranean environments), or in covered sites which has more humid soils, but much less light than the plant requires

(Garrido et al., 2007), the environmental conditions overwhelm any effect of the elaiosome, with the result that the effect of elaiosome on emergence is expressed only in partially, intermediate conditions. Interestingly, this explanation is reinforced by its regional congruence, as it appears at all studied localities in the same sense.

Finally, regarding seedling survival, we determined that the presence or absence of an elaiosome attached to the seed does not modify seedling survival under any set of environmental conditions. The advantages conferred by the elaiosome thus seem to be limited to germination, emergence and predation avoidance.

5. Concluding remarks

Many advantages have been traditionally attributed to myrmecochory (Beattie and Hughes, 2002), but the fate of the seeds after ant manipulation has rarely been examined (see Manzaneda et al., 2005). Here, we have shown that ants, by removing the elaiosome, may prevent seeds from long-term predation (probably the major seed drain in this species) and enhance seedling emergence, at least at intermediate environments. Conjunction of these 'delayed' effects of the elaiosome makes this dispersal mode a highly effective one, underlining its key role in the evolution of ant–plant interactions. Despite this, to our knowledge, these additional advantages conferred by the elaiosome have not been yet described by any study and deserve further investigation if we are to know the real ecological and evolutionary effects of this structure.

It has been proposed that the emergence-enhancing effect of the elaiosome removal is a 'secondary benefit' of ant dispersal (Imbert, 2006, and references therein). We disagree with the view of considering elaiosome removal as the 'cast actor' in the performance of myrmecochory. Because nothing is achieved with seed removal if dispersed seeds lose germination viability or are eventually consumed, and both risks increase with the long-term maintaining of the elaiosome, we suggest that elaiosome removal provides a delayed but primary service in this kind of mutualism. This delayed effect of elaiosome removal, together with the tendency to decrease predation, reinforces the perspective from which this dispersal mechanism is nowadays considered, making it even more ecologically and evolutionarily fascinating.

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