Adaptive advantages of myrmecochory: the predator-avoidance hypothesis tested over a wide geographic range

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The predator-avoidance hypothesis states that once released from the parent plant, myrmecochorous seeds are rapidly taken by ants to their nests, where they are protected from predators. Previous studies conducted to test this hypothesis have frequently neglected two major aspects necessary for its verification: 1) the influence of processes acting after the seed release and 2) the spatial evenness of such processes. Thus, large-scale variations in the mechanisms acting beyond seed release, and possibly influencing seed escape from predators, remain poorly documented. Here, we present the results of a post-dispersal seed-removal experiment on the myrmecochorous herb *Helleborus foetidus*, aimed at verifing the predator-avoidance hypothesis by considering two key post-release aspects of seed fate: seed destination (dispersed or nondispersed) and seed burial (buried or not buried). Experiments were performed in four different regions in the Iberian Peninsula. After three days of exposure of seeds to the main predator (fieldmice *Apodemus sylvaticus*), ca 30% of the seeds were removed. Seed destination affected the proportion of seeds escaping predation, but the sign, magnitude and statistical significance of the effect varied among the geographical regions. In the southern region (Cazorla), seeds dispersed in ant nests or intermediate destinations suffered scarcely any predation, but seeds under reproductive-age plants experienced losses ca 50%. Conversely, in the northern region (Caurel), seeds in nests suffered significantly greater losses than seeds under plants or intermediate destinations, suggesting that nests were especially unsafe destinations. Seed burial had a strong impact on seed escape from predators, and its effect was highly consistent among geographical regions. In view of the consistency of its effect at different spatial scales, seed burial was a more general mechanism for predation avoidance than seed relocation to ant nests, which was habitat- and/or ant-species-dependent. Our results thus only partially support the predator-avoidance hypothesis for the evolution of myrmecochory.

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Myrmecochory (seed dispersal by ants) is one of the best-known cases in nature of directed dispersal, i.e. that type of seed dispersal in which seeds are disproportionately deposited in favourable locations (Howe and Smallwood 1982, Hanzawa et al. 1988, Wenny 2001, Wang and Smith 2002). The benefits of seed dispersal by ants are determined by the specific seed arrival environ-

ment (the nest) rather than simply the removal of seeds away from their parents (but see Andersen 1988, Whitney 2002). Arrival-related benefits of myrmecochory and the adaptive advantages for plants are relatively well known (see recent reviews by Beattie and Hughes 2002, Gorb and Gorb 2003). Of these potential benefits, the predator-avoidance hypothesis – i.e. a presumed

selective advantage of the increased escape from post-dispersal predators of the seeds deposited in ant nests – has received much attention. Predator avoidance has been shown to be a significant benefit of myrmecochory in diverse systems in the northern hemisphere (Turnbull and Culver 1983, Gibson 1993, Espadaler and Gómez 1996, 1997, Ruhren and Dudash 1996, Ohkawara et al. 1996, Boyd 2001, but see Kjellsson 1985, Guitián et al. 2003), and also in the South African fynbos (Bond and Breytenbach 1985, Slingsby and Bond 1985, Christian 2001, Christian and Stanton 2004). However, in habitats like dry sclerophyll woodland in Australia, where the myrmecochore flora is better represented (Berg 1975), the benefit of predator avoidance for plants has been verified only partially (Westoby et al. 1991, but see Auld and Denham 1999).

Some authors (Heithaus 1981, Smith et al. 1986, Bennett and Krebs 1987, Ruhren and Dudash 1996, Gorb and Gorb 2003) have argued that seed predation constitutes the main ecological driving force contributing to the natural selection of plant species towards myrmecochory. This is because seed predation is not only a key process in regeneration and/or spatial structuring of plant communities (Crawley 2000, Wang and Smith 2002, Rey et al. 2002), but also because predators may act as agents of natural selection that influence the evolution of specific seed traits, e.g. shaping the features of seed dispersal syndromes (Ruhren and Dudash 1996, Benkman 1999, Hulme and Benkman 2002, Benkman et al. 2003, Schöning et al. 2004).

Much previous work involving ants, elaiosome-bearing seeds and post-dispersal seed predation has focused on the potential interference that predators may cause during the earlier phases of the ant-seed dispersal process, and has sought to quantify the impact of predators relative to ant-dispersers on seed removal rate during the seed-release period (Heithaus 1981, Turnbull and Culver 1983, Smith et al. 1989, Ruhren and Dudash 1996). Certainly, this approach facilitates investigation of one of the main mechanisms involved in the escape of elaiosome-bearing seeds from predators, namely the timing of seed dispersal over the seed-release period. In other words, this approach allows testing of the first component of the predator-avoidance hypothesis: seeds are rapidly removed by the ants, preventing their discovery by post-dispersal predators. Although consideration of this component is crucial for testing the predator-avoidance hypothesis, it may not to be sufficient for its full corroboration. Specifically, predators may interfere with the ant-dispersal mutualism after seed release, in the ant nests to which the seeds are transported, however, this possibility has been little explored. In other words, a second component of the predator-avoidance hypothesis is rarely tested: whether or not ant nests are safe sites for dispersed seeds. In fact, the mechanisms that might make a nest a safe microsite

for seeds and/or the processes that might deter predators, like seed processing and handling by ants, are all neglected. These aspects are particularly important for seeds that show dormancy and stay in nests or nest middens for long periods. Thus, little information is available concerning the effects on seed predation risk of 1) seed relocation in ant nests or waste middens (though see O'Dowd and Hay 1980, Hanzawa et al. 1988, Hughes and Westoby 1992a, Boyd 2001); 2) seed burial (though see Heithaus 1981, Beattie and Culver 1982, Bond and Breytenbach 1985, Hughes and Westoby 1992a, Christian and Stanton 2004); 3) the removal of the elaiosome (though see Heithaus 1981, Bond and Breytenbach 1985, Hanzawa et al. 1985, Boyd 2001, Christian and Stanton 2004); or 4) fine-scale spatial distribution of seeds (though see Hughes and Westoby 1992b, Auld and Denham 1999).

Another problem in evaluations of the predator-avoidance hypothesis is that the vast majority of the studies have been conducted on local frameworks (but see Gibson 1993). This is especially significant because the study of the evolution and maintenance of species interactions requires a multipopulational approach (Thompson 1988, 1994, 1999, Thompson and Cunningham 2002). Thus, in the particular case of ant-plant interactions, some authors have suggested that the outcome of the interaction may vary over large geographic regions (Cushman and Beattie 1991, Bronstein 1994, Cushman et al. 1998, Beattie and Hughes 2002). However, despite the fact that the ant-seed dispersal process is highly generalist (Handel and Beattie 1990), and that the guild of ant-dispersers varies in space (Garrido et al. 2002), hardly any experimental studies have evaluated the ecological and adaptive significance of the ant-seed dispersal process on wide spatial scales and/or along environmental gradients (Beattie and Hughes 2002).

Here, we present the results of a wide-scale post-dispersal seed-removal experiment, aimed at testing the predator-avoidance hypothesis in the ant-dispersed perennial herb *Helleborus foetidus* (Ranunculaceae). Fedriani et al. (2004) have recently shown geographical variation in the potential of mice to interfere with the ant-seed mutualism in *H. foetidus*, suggesting that seed escape from post-dispersal predators varies among geographical regions. Unlike most other previous studies, Fedriani et al.'s study was performed over a large geographical area; however, it considered only the first component of the predator-avoidance hypothesis (i.e. protection from predators during seed release period). Possible large-scale variations in the mechanisms acting beyond seed release (i.e. the second component of the hypothesis) remain unexplored. In the present paper we consider two central aspects of post-release ecology, along a broad geographical gradient: seed destination and seed burial. More specifically, we address the

following questions: 1) do ant nests constitute a safer microsite for *H. foetidus* seeds than other potential destinations? 2) If so, how consistent is the "safeness" among regions? 3) Is seed burial an effective mechanism for avoiding predation? 4) If so, does this effectiveness vary depending on seed destination or geographical region?

Materials and methods

Study system and sites

Helleborus foetidus (Ranunculaceae) is a perennial herb distributed throughout western Europe (Werner and Ebel 1994). In the Iberian Peninsula, it is generally found in clearings, in patchy scrublands, forest edges, and the understory of deciduous and mixed forest. Plants consist of one or several ramets, each of which produces a single terminal inflorescence after several seasons of vegetative growth. Flowering takes place from January to March, and each inflorescence yields 25–100 flowers (Herrera et al. 2001). Flowers have 1–5 carpels, each of which develops 10–12 elaiosome-bearing seeds (Garrido et al. 2002). Diaspore (seed plus elaiosome) fresh mass ranges from 5 to 23 mg. The elaiosome is white and soft, and comprises ca 3–15% of the total diaspore fresh mass. Fruit maturation and seed shedding take place in June–July. Ants (mostly of the genera *Lasius*, *Formica*, *Camponotus* or *Aphaenogaster*, Garrido et al. 2002) are attracted by the elaiosome, and remove the diaspore once it has fallen on the ground, or they may climb the plant to gather diaspores directly from dehiscing fruits. The diaspores are carried to the nest, where the elaiosome is removed and eaten; then, depending on the ant species, the intact seed may be discarded within the nest or on a midden on the surface. *Helleborus foetidus* seeds have a two- to three-year dormancy period (Herrera et al. 2002, Garrido 2003). The main post-dispersal consumers of *H. foetidus* seeds within the Iberian Peninsula are field mice *Apodemus sylvaticus* (Herrera et al. 2002, Fedriani et al. 2004). Field mice act exclusively as seed predators (not dispersers), although their impact on seed numbers may vary significantly among geographical regions (Fedriani et al. 2004). Systematic sampling with pit-fall traps showed that other seed predators such as granivorous ants or ground beetles were rare or absent at our study sites (Manzaneda unpubl.).

The present study was conducted during June–July of 2003, coinciding with the *H. foetidus* seed shedding period, at seven populations in four separate geographical regions in the Iberian Peninsula (Fig. 1). These populations were chosen because they cover much of the natural range of *H. foetidus* in the Iberian Peninsula, and are known to comprise contrasting ant communities (Garrido et al. 2002), representing besides diverse

ecological conditions in which this species occurs (Herrera et al. 2001). In addition, three of the four regions included in this study coincide with those studied by Garrido et al. (2002) and Fedriani et al. (2004). In the Caurel region both populations were in sites with dense bracken (*Pteridum aquilium*) coverage, but one site was located within a *Pinus sylvestris* plantation at 1200 m a.s.l. while the other, ca 2 km away, was located in an open successional scrubland. In the Peña Negra region, the two populations (ca 2 km apart) were located within an oak-dominated deciduous forest (*Quercus pyrenaica*) at 1400 m a.s.l. In the Cazorla region, the two populations (ca 5 km apart) were located in mixed forest (*Pinus nigra* and *Quercus rotundifolia*) at 1300 m a.s.l. Finally, in the Mágina region the single population was located in an open area with a rather sparse scrub layer of *Berberis hispanica*, *Crataegus monogyna*, *Echinospartum boissieri* and *Erinacea anthyllis* at 1650 m a.s.l.

Experimental design and procedure

To evaluate the predator-avoidance hypothesis at the post-release stage, in each population we performed a factorial experiment with two factors, seed destination and seed burial. The factor seed destination had three levels: seed carried to nest ("nest"), seed deposited between plant and nest ("midway"), and seed not removed ("nondispersed"). The intermediate level corresponds to seeds dropped by ants, often a significant proportion (Gorb and Gorb 1999). The factor seed burial had two levels: seed buried ("buried"), and seed exposed on surface ("surface"). On the basis of ant disperser census (Manzaneda unpubl.), we chose active nests of ant species with the highest seed removal rates in each population. A total of 78 nests belonging to five different ant species were included in the experiment (between 7 and 15 nests per population, depending on nest density and/or the difficulty of finding them; ant species were non-sympatric, see Table 1). The experimental units consisted of seed depots allowing seed removal by mice (the post-dispersal predator) but preventing removal by ants. Each depot consisted of 20 fresh *H. foetidus* diaspores ("seeds" hereafter) glued (using Loctite©, which has low-odour when dry) to the sides of a 10 × 10 cm square of fibre-glass mesh that was nailed to the ground. This procedure of exclusion has been shown to be effective since rodents can easily remove the seeds by chewing the fibre-glass threads, while ants and other potential removers like ground beetles or birds are unable to do so (Alcántara et al. 2000, Rey et al. 2002). The six treatment combinations, corresponding to our 3 × 2 experimental design were achieved as follows. 1) Nest+Surface: the seed depot was placed in the nest's midden (though for one ant species, *Lasius fuliginosus*, no defined midden was
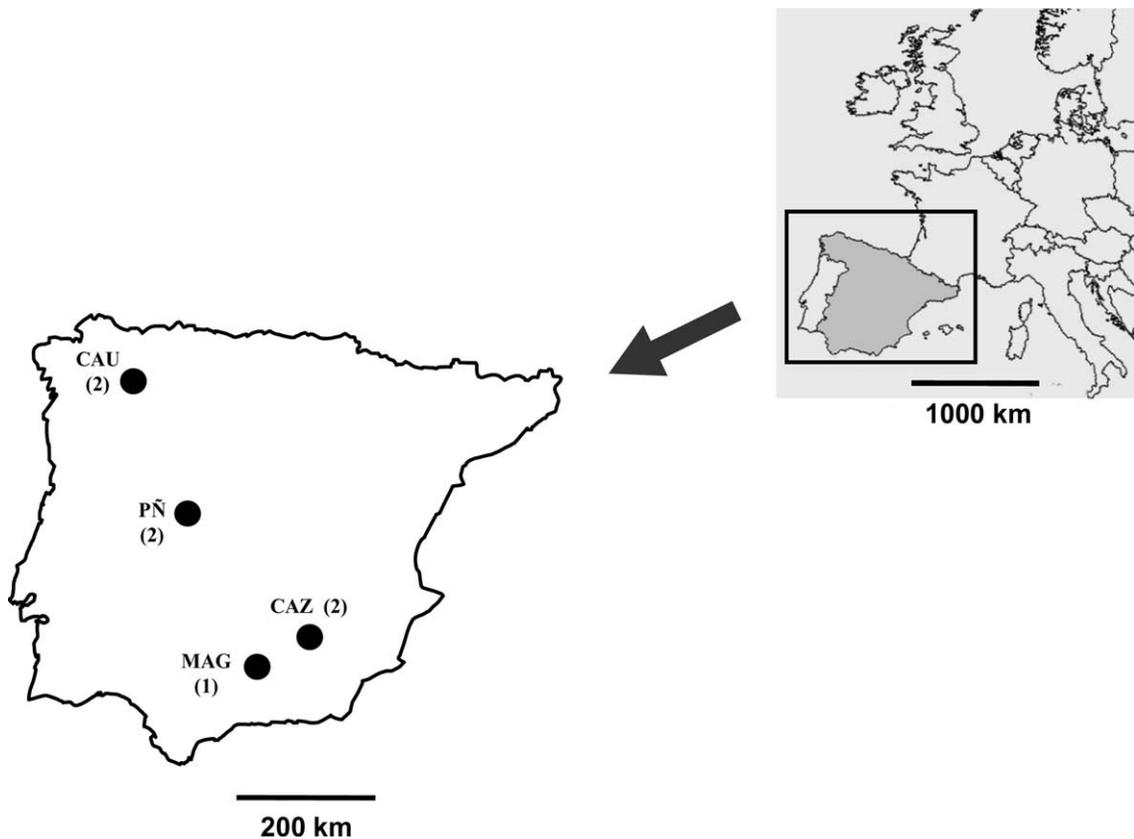
Fig. 1. Map of the Iberian Peninsula (left), showing the location of the four study regions. The figures in brackets show the number of *H. foetidus* populations studied in each geographical region. Regions were: Sierra del Caurel (CAU: 42°39′N, 7°7′W); Sierra de la Peña Negra (PÑ: 40°28′N, 5°20′W); Sierra de Cazorla (CAZ: 37°56′N, 2°52′W); Sierra Mágina (MAG: 37°44′N, 3°28′W). The two most distant regions (Caurel and Mágina) were ca 760 km apart, while the two closest regions (Mágina and Cazorla) were only ca 85 km apart.

evident and thus seed depots were placed close to the nest entrances). 2) Nest+Buried: the seed depot was buried to a depth of ca 2–3 cm in the midden. 3) Nondispersed+Surface: the seed depot was located directly under the reproductive-age plant closest to the nest (i.e. each selected nest had an associated plant, in all cases within the dispersal range of that particular ant-disperser species; Table 1). 4) Nondispersed+Buried: the seed depot was buried to a depth of ca 2–3 cm directly under the associated plant. 5) Midway+Surface: the seed depot was located halfway between the nest and its associated plant. 6) Midway+Burial: the seed depot was buried to a depth of ca 2–3 cm halfway between the nest and the associated plant. In all cases, we manually removed all other seeds that we were able to locate in each microsite. Each set of six treatment combinations

Table 1. Some ecological characteristics of the ant species considered in each geographical region and population of *H. foetidus*. An estimate of the abundance of fieldmouse *Apodemus sylvaticus* in each population is also given.

Region	Species	No. nests found*	Midden	Midden area (m²)†	Midden substrate‡	Dispersal distance (m)∞	Fieldmouse abundance¶
Caurel	*Lasius fuliginosus*	12	Diffuse	–	Pine litter	2.48±1.02	1.45
	Formica lugubris	7	Discrete	0.391±0.120	Soil and plant matter	3.53±3.44	1.85
Peña Negra	*Lasius emarginatus*	7/11	Discrete	0.028±0.007	Soil	1.29±0.89	12.65/3.1
Cazorla	*Camponotus cruentatus*	15/13	Discrete	0.607±0.25	Soil	6.07±3.40	21.4/3.85
Mágina	*Aphaenogaster senilis*	13	Discrete	0.038±0.007	Soil and plant matter	3.05±1.41	1

* Number of nest (replicates) located and used in the experiment at each population.
† Mean±SD.
‡ In case of *L. fuliginosus* substrate of the main nest entrance. In all cases the presence of waste material was corroborated.
∞ Mean±SD. From Manzaneda (unpubl.).
¶ Mean index of fieldmouse abundance (see Materials and methods for details).

constituted a block, which was replicated 7–15 times per population (depending on the number of nests found; see above and Table 1). Within each population, the distance between adjacent blocks was determined by the specific spatial distribution of nests of each species, but was always ≥2 m. Within each block, seed depots were located at a minimum distance of 15 cm from each other, while the maximum distance between depots depended on plant-nest distance, which ranged from 1.2 to 10 m (Table 1). All seed depots (9360 seeds in N = 468 depots) were checked within two hours of dawn on three consecutive days (see Hulme 1994), recording the number of seeds remaining. Afterwards, the cumulative proportion of seeds remaining at the end of the 3-d period was used as dependent variable. This time span corresponds with the maximum time that, in our study sites, seeds remain available to mice before removed by ants (Fedriani et al. 2004).

In addition, fieldmice abundance at each of the seven sites was estimated on the basis of live-trapping in Sherman traps baited with peanut butter (N = 40–60 traps per population), as $100 \times$[number of individuals trapped] divided by [number of night-traps]. Two trapping surveys were conducted in each population, one just before the seed-release period (end of May) and the other at the seed-release peak (end of June); neither survey overlapped in time with the removal experiments. Traps were activated at dusk and checked at dawn, for up to six consecutive nights. Both trapping sessions as experiments were conducted out of full moon days. Captured mice were released immediately after identification.

Data analysis

To evaluate the significance of the effects of geographical region (Region), seed destination and seed burial on post-dispersal predation, we fitted a generalized linear mixed model using SAS macro GLIMMIX (Littell et al. 1996), considering the response variable (i.e. proportion of seeds remaining after 72 h of exposure to fieldmice) to have a binomial distribution, and with a logit link function (see Herrera 2000). Region, seed destination, seed burial and all possible two- and three-way interactions among these three main factors, were included as fixed-effect factors in the model. Population (nested within Region) and block (nested within Population and Region) were considered as random-effect factors (the proper manner to treat nested effects, see Bennington and Thayne 1994). When a significant interaction between main effects was detected, we carried out "tests of simple main effects" (which allow the effects of a given factor to be explored at each level of the other factors, Schabenberger et al. 2000), using the SLICE option in the LSMEANS statement of the MIXED

procedure (Littell et al. 1996). The LSMEANS statement provided model-adjusted means and standard errors (back-transformed from logit scale, using the appropriate Taylor's series approach, Littell et al. 1996).

Results

In all populations the only rodent species captured was *Apodemus sylvaticus*. Although fieldmouse abundance varied among populations and geographical regions (Table 1), we did not find any overall correlation between the proportion of seeds remaining and fieldmouse abundance (Spearman correlation $r_s = -0.1071$, p = 0.82, N = 7). After three days of exposure to predators, ca 30% of the seeds were removed. The overall proportion of seeds remaining did not vary among geographical regions (Table 2): adjusted means (±1 SE) were, 0.81 ± 0.007, 0.89 ± 0.007, 0.76 ± 0.002, 0.90 ± 0.046 for Caurel, Peña Negra, Cazorla and Mágina, respectively.

As a main effect, seed destination did not affect the proportion of seeds remaining; however, the interactive effect of Destination × Region was statistically significant (Table 2), indicating that there was a destination effect but that its sign and/or magnitude depended on the geographical region. Tests of simple main effects showed that Caurel and Cazorla were the regions in which seed escape from predators depended on seed destination (Table 3), although with opposite sign (Fig. 2): in Cazorla, seeds in ant nests or midway sites suffered scarcely any predation, but seeds depots under reproductive-age plants (i.e. nondispersed) experienced losses of nearly 50% (Fig. 2); conversely, in Caurel, seeds in nests suffered significantly greater losses than seeds under plants or in midway sites (Fig. 2), suggesting that ant nests were especially unsafe destinations in the Caurel. In the other two geographical regions (Peña Negra and Mágina), seed destination did not affect the proportion of seeds remaining (Table 3, Fig. 2). Like-

Table 2. Results of the Generalized Linear Model testing the effects of geographical region (Region), seed destination (Destination) and seed burial (Burial) on the proportion of seeds remaining after three days of exposure to fieldmice.

Effects	DF	F	p
Region	3,3	0.21	0.884
Destination	2,370	2.30	0.102
Burial	1,370	105.45	<0.0001
Region × Destination	6,370	5.14	<0.0001
Region × Burial	3,370	1.83	0.141
Destination × Burial	2,370	0.76	0.467
Region × Destination × Burial	6,370	0.49	0.816

Random effects		Z	p
Population (Region)		0.98	0.163
Block (Population (Region))		4.37	<0.0001

Table 3. Tests of simple main effects (interaction slices) for the effect of seed destination (Destination) on the proportion of seeds remaining within each geographical region (Region).

Effects	Region	DF	F	p
Destination	Caurel	2,370	5.00	0.0072
Destination	Cazorla	2,370	13.54	<0.0001
Destination	Mágina	2,370	0.47	0.624
Destination	Peña Negra	2,370	0.46	0.632

wise, the proportion of seed remaining did not vary significantly among populations within geographical regions, but differences among blocks within populations were statistically significant (Table 2), indicating that escape from predators varied at the micro-scale level.

Seed burial had a strong impact on seed escape from post-dispersal predators (Table 2): the proportion of seeds remaining (adjusted mean ± 1 SE) was 0.63 ± 0.05 and 0.95 ± 0.11 for seeds exposed on the surface and buried seeds respectively. Furthermore, this effect of burial was highly consistent among geographical regions (Fig. 3), as shown by the non-significance Region $\times$ Burial interaction (Table 2), and was independent of

seed destination, as indicated by the non-significance of the Destination $\times$ Burial interaction (Table 2).

Discussion

It is commonly accepted that once myrmecochorous seeds reach the ant nest their chances of escaping from predation are enhanced (Beattie 1985, Stiles 2000). However, our results do not wholly support this traditional view. The enlargement of the spatial framework in our study of seed fate in ant-seed dispersal has revealed that ant nests can act as safe microsites but also as neutral-microsites or, more surprisingly, as risky-microsites for *H. foetidus* seeds (Fig. 2). Our results are in line with the general view expressed by various authors (Thompson 1994, 1999, 2002, Cushman et al. 1998), that the source of the variations in the outcome of species interactions arise from variations in the geographic structure within which these interspecific interactions take place. Our results are also in accordance with the view that the adaptive advantages of seed relocation to nests and/or waste middens are rather
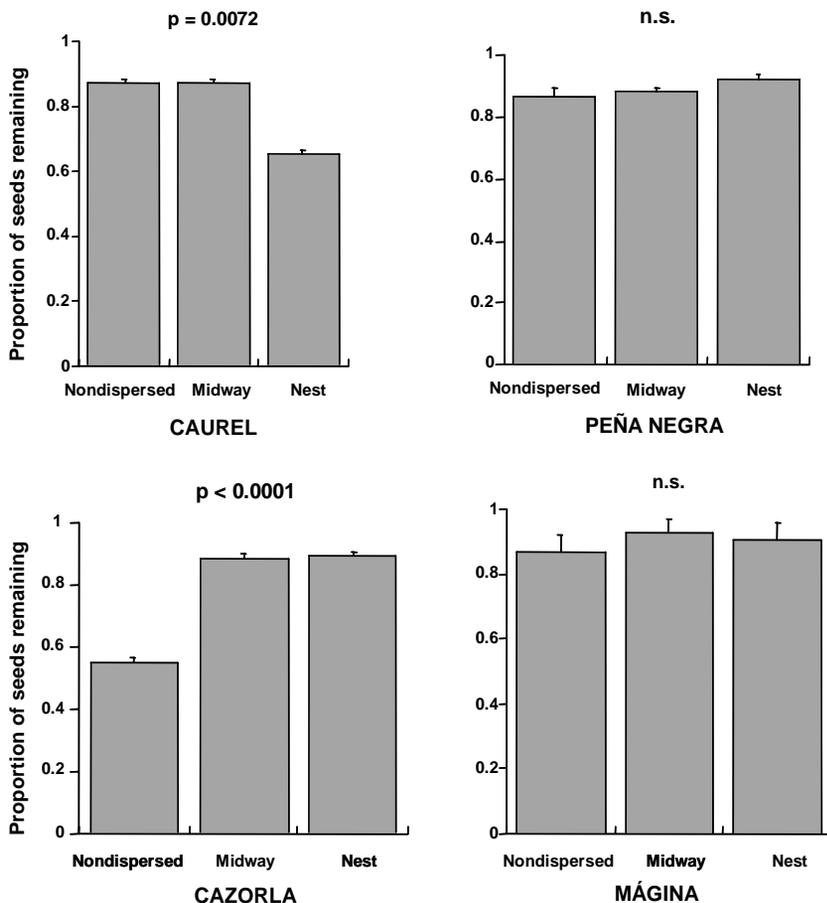

Fig. 2. Adjusted mean proportion of seeds remaining (± 1 SE) in each geographical region and for each seed destination after three days of seed exposure to predators (fieldmice). In each plot, significance p-values come from tests of simple main effects (interaction slices) for the significant interaction Region $\times$ Destination (see Table 3).
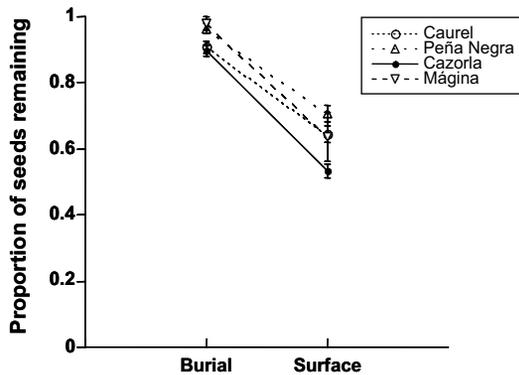
Fig. 3. Proportion of seeds remaining (adjusted mean ± 1 SE) in each geographical region and each burial treatment.

species-specific and habitat-dependent (Beattie 1985, Handel and Beattie 1990, Hughes and Westoby 1992a, Espadaler and Gómez 1996). In others words, our results suggest that the magnitude or even sign of one particular advantage of a given interaction (here, post-dispersal avoidance of seed predation) may vary markedly depending on the interacting species (here, the ant species) and on habitat.

Because the probability of seed escape from predation was not correlated with fieldmouse abundance (lack of correlation between the abundance of predators and the intensity of post-dispersal seed predation seems to be frequent: Heithaus 1981, Hulme 1994, Manson and Stiles 1998, Rey et al. 2002), other factors are presumably involved in this pattern of variation. One possibility is that the observed among-region and among-microsite variations in post-dispersal seed predation reflect variations in habitat structure: (e.g. plant cover, density and type of ground cover, or distance from the forest edge), that may impinge on the foraging behaviour of rodents (Myster and Pickett 1993, Manson and Stiles 1998, Alcántara et al. 2000, Schreiner et al. 2000, Taraborelli et al. 2003, Fedriani 2005). In turn, variations within a particular habitat in the spatial distribution of the different ant-disperser nests with respect to the spatial structure of the vegetation, probably explain the observed differences among the ant-disperser species in effectiveness for preventing predation. Similar results have been obtained previously for myrmecochorous species in semi-arid environments with a patchy vegetation structure (O'Dowd and Hay 1980). These authors found that ant nests (typically in open sites) were infrequently located within rodent foraging areas (typically under plant cover). This implies that seeds carried to ant nests avoid predation simply because they were located outside of the foraging area of the rodents. In line with this, in the present study the only region in which ant nests (belonging to *Camponotus cruentatus*) constituted a safe microsite for *H. foetidus* seeds was Cazorla (Fig. 2). In both populations in this region,

C. cruentatus nests are located in open areas at the edge of mixed forest or in locations partially covered by canopy (unpubl.), whereas *H. foetidus* plants are located close to shrubs within the understory of the mixed forest. This "uncoupling" between the locations of *C. cruentaus* nests and *H. foetidus* plants may partly explain why mice, which avoid open microsites (Fedriani 2005), do not forage near *C. cruentatus* nests.

Although plant cover may thus be relevant, it was probably not the only factor affecting seed predation patterns. Factors such as the speed of seed removal by ants, dispersal distance, and variations in the availability of alternative food for rodents (Veech 2001, LoGiudice and Ostfeld 2002), may also have been partially responsible for the observed variations in the pattern of post-dispersal seed predation. Fedriani et al. (2004) showed that *H. foetidus* seeds were removed by ants more rapidly in Caurel than in Cazorla. They also found that in Cazorla mice were more likely to interfere with the ant-seed dispersal mutualism; unfortunately they did not consider this aspect in their study of Mágina and Peña Negra. This would imply that rodent foraging patterns reflect some knowledge of seed availability. Thus, if ants rapidly remove seeds from under plants, as in Fedriani et al.'s study in the Caurel region, mice may opt to forage in ant nests. In contrast, if seed removal by ants is slow (as in Cazorla, Fedriani et al. 2004), mice may opt to forage directly beneath *H. foetidus* plants rather than in nests or nest middens, since seeds stay longer beneath plants. This explanation seems plausible since many ant species re-locate the seeds on a waste midden at the ground surface once they have been processed (Beattie 1985, Gómez and Espadaler 1998), creating a spatially and temporal aggregated source of resources that is easily detectable by mice (Crawley 2000).

Our experimental design allowed us to assess whether seed escape from predators results merely from leaving the parent plant microsite, or is a particular benefit of reaching the ant nest. Our results in fact suggest that seed escape from predators may be more closely related to dispersal distance than to actual arrival at the ant nest. Thus, in Cazorla, the only region in which ant nests were safe microsites, we did not detect any differences in the proportion of seeds remaining between the Midway seed destination and the nest seed destination (Fig. 2), which suggests that escape from seed predation results from the displacement of seeds away from the parent plant, so that dispersal distance plays a central role in protection against predation.

In contrast with the large-scale variations observed in the escape of *H. foetidus* seeds from predation, our results show that the effect of burial on seed escape was strongly consistent across all spatial scales considered in this study (Fig. 3). This suggests not only that seed burial is a highly effective and general mechanism for avoiding predation in many different ecological conditions, but

also that it is a more efficient mechanism for avoiding predation than seed relocation to ant nests or mechanisms related to the timing of seed release (whose effects are habitat- and/or species-dependent, Fig. 2, Fedriani et al. 2004). Seed burial has been suggested to be a major determinant of seed escape from post-dispersal predators (Hulme 1994, Crawley 2000). Our results support this view and are in line with previous studies that have pointed to seed burial as a major mechanism of predator avoidance via myrmecochory (Heithaus 1981, Beattie and Culver 1982, Bond and Breytenbach 1985, Christian and Stanton 2004). Accordingly, predator avoidance will be an adaptive advantage of myrmecochory so long as the ant-disperser guarantees high rates of seed burial. However, at least two constraints may limit the efficiency of this mechanism. First, although seeds are generally discarded in the nest after processing, some ants return the seeds to the surface, presumably making them more vulnerable to predators (Gómez and Espadaler 1998). Second, the depth at which seeds are buried may constitute a trade-off between the probability of escaping from predators and the probability of emerging in optimal conditions (Bond and Breytenbach 1985, Christian and Stanton 2004). The latter is particularly relevant since it has been suggested recently that the optimum burial depth for germination and emergence is strongly species-specific (Christian and Stanton 2004). Unfortunately, studies on seed fate at the species level are extremely rare (but see Hughes and Westoby 1992a, b), and insufficient to assess which ants or guild of ants provide protection from seed predators, and which do not. In our system, not all the ant species show the same dispersal behaviour. For example, it is known that *Aphaenogaster* returns the seeds to the surface once the elaiosome is consumed (Gómez and Espadaler 1998, unpubl.), while the proportion of seeds discarded to *Camponotus cruentatus* nest middens is relatively small (Manzaneda unpubl.). No data concerning burial depth and seed-seedling survival are available for our system, and certainly continued research is needed to confirm that predator avoidance due to seed burial is indeed a selective benefit of myrmecochory. Nevertheless, given the strong impact that post-dispersal seed predation may have on *H. foetidus* recruitment (Garrido 2003), and the strong effect of seed burial on seed escape from predation (Fig. 3), it may be expected that natural selection will favour those features of seeds that promote seed burial. Such features presumably include, elaiosome-related traits (seeds with elaiosome have a greater chance of being removed by ants and therefore of being buried, see e.g. Boyd 2001, Garrido et al. 2002) and seed size (smaller seeds have a greater chance of escaping predation once buried, Hulme 1994).

Finally, and in contrast with previous studies of the same ant-dispersal system (Garrido et al. 2002, Fedriani et al. 2004), our results do not indicate "ecological equivalence" (sensu Zamora 2000) among the ant-disperser assemblages from different regions. Indeed, as mentioned above, one of the main conclusions that can be drawn from our results is that the effect on seed predation risk of relocation to the ant nest seems to be related to the precise microhabitat in which the nest of particular ant species were located. This idea is consistent with the fact that in Caurel, the only geographical region with two different ant species, the consequences of seed relocation on ant nests were different. Thus, the overall proportion of seeds remaining in *F. lugubris* nests was nearly two-fold higher than the proportion of seeds remaining in *L. fuliginosus* nests (mean ± 1 SD; 16.00 ± 8.99, 8.54 ± 7.04, for *F. lugubris* and *L. fuliginosus* respectively; U Mann-Whitney test, $Z = 2.91$, $p = 0.0036$). Hence, more studies are needed on the specific outcomes of ant dispersal beyond the seed removal and seed predation stages, (i.e. germination, seedling survival, etc.) in order to assess whether ant-dispersal systems indeed show such functional equivalence. However, our results are in line with previous findings indicating that ant-seed interactions show a mosaic-like geographic structure. For example, we found that the interaction had positive consequences for the plant in the Cazorla region, but negative consequences in the Caurel region. This suggests the existence of local maladaptations between *H. foetidus* seeds and their dispersers, which is an expected result of the coevolutionary process (Thompson et al. 2002).

In conclusion, the present results underline the importance of considering a wide spatial framework in studies aimed at elucidating the ecological and evolutionary significance of interspecific interactions, which has rarely done, especially in ant-plant interactions studies (Cushman et al. 1998, Beattie and Hughes 2002).

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