

Assessing ecological specialization of an ant–seed dispersal mutualism through a wide geographic range

ANTONIO J. MANZANEDA^{1,3} AND PEDRO J. REY²

¹Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas,
Avda. María Luisa s/n, E-41013, Sevilla, Spain

²Departamento Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén
Paraje las Lagunillas s/n, E-23071, Jaén, Spain

Abstract. Specialization in species interactions is of central importance for understanding the ecological structure and evolution of plant–animal mutualisms. Most plant–animal mutualisms are facultative and strongly asymmetric. In particular, myrmecochory (seed dispersal by ants) has been regarded as a very generalized interaction. Although some recent studies have suggested that only a few ant species are really important for dispersal, no rigorous measurement of the specialization in ant–seed dispersal mutualisms has been performed. Here, we use individual plants as basic units for replication to investigate the generalization–specialization of the herb *Helleborus foetidus* on its ant dispersers over a considerable part of its geographical range. We define generalization in terms of diversity components (species richness and evenness) of the ant visitor that realizes dispersal by removing diaspores. We obtain truly comparable values of ant visitor diversity, distinguishing among different functional groups of visitors and identifying incidental visitors and real ant dispersers. Using null model approaches, we test the null hypothesis that ant-mediated dispersal is a generalized mutualism. At least two premises should be confirmed to validate the hypothesis: (1) diaspores are dispersed by multiple ant-visitor species, and (2) diaspore dispersal is significantly equitable. Though up to 37 ant species visited diaspores across 10 populations, only two large formicines, *Camponotus cruentatus* and *Formica lugubris*, were responsible for the vast majority of visits resulting in dispersal in most populations and years, which strongly suggests that ant seed dispersal in *H. foetidus* is ecologically specialized. Interestingly, specialization degree was unrelated to dispersal success across populations. Our study offers new insights into the spatiotemporal dynamics of myrmecochory. We propose the existence of an alternative scenario to extensive generalization. In this new scenario, generalization is replaced by ecological specialization, which is determined by the intrinsic traits of the plant species rather than by the ecological context in which the interaction takes place.

Key words: ant–plant mutualism; ecological generalization; evenness; geographic variation; hellebore; *Helleborus foetidus*; myrmecochory; null models; seed removal; species richness estimation.

INTRODUCTION

Ecological specialization (one or few partner species) or its opposite, generalization (high number or diversity of partner species), has long been a central focus in the study of plant–animal interactions. Historically, specialization was invoked as a fundamental process to explain the evolution of phenotypic traits in the plants and animals involved in mutualistic interactions (Darwin 1862, Nilsson 1988, Herrera 1996, Pellmyr 2002) and to drive coevolution (Janzen 1966, Pellmyr et al. 1996, Pellmyr 2003, Villensen et al. 2004), diversification, and speciation within communities (Ehrlich and Raven 1964, Armbruster and Baldwin 1998, Price and Wagner 2004).

Recently, it has also been proposed that the degree of specialization in species' interactions can play a fundamental role in affecting the structuring of mutualistic and antagonistic assemblages within a community (e.g., Bascompte et al. 2003, 2006).

Most plant–animal mutualisms have frequently been assumed to involve facultative interactions that are weak in intensity and are asymmetric (Jordano 1987, Howe 1993, Olesen and Jordano 2002, Vázquez and Aizen 2004, Bascompte et al. 2006, but see Okuyama and Holland 2008). Recent criticisms, however, have been raised about how generalization–specialization patterns are habitually studied, questioning the conclusions reached in favor of generalization (see Ollerton and Cramer 2002, Kay and Schemske 2004, Herrera 2005, Moeller 2005).

Although well-documented examples exist in nature of highly specialized mutualisms between ants and some plants species (Janzen 1966, Beattie and Hughes 2002),

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³ Present address: Department of Biology, Duke University, P.O. Box 90338 Durham, North Carolina 27708 USA.
E-mail: antonio.manzaneda@duke.edu

plant–ant mutualisms are usually characterized as generalized and facultative relationships (Bronstein 1994, Rudgers and Strauss 2004, Guimarães et al. 2006, Ness et al. 2006). It has been suggested that the commonness of the generalized ant–plant mutualism is due to: (1) the huge variation over space and time in ant assemblages and in the variable benefits provided by different ant species (Rudgers and Strauss 2004, Manzaneda et al. 2005, Ness et al. 2006), (2) the nonselective yet high attractiveness of the plant rewards offered to ants (e.g., extra-floral nectaries or elaiosomes [Beattie 1985]), and (3) the omnivorous and generalist nature of ants. These three particularities, together with the fact that dispersal-related rewards of myrmecophilous plants do not require, in general, any ant pre-adaptation or fine adjustment, determine the low specificity of ant–plant mutualisms. Within ant–plant interactions, myrmecochory (seed dispersal by ants) is thought to be a very generalized interaction (Beattie and Hughes 2002); i.e., diaspores are dispersed by multiple ant visitor species. However, some evidence exists that only some particular species guilds (e.g., large-bodied species [Ness et al. 2004, Manzaneda and Rey 2008]), or even one or a few ant species, may be really important for dispersal (Gove et al. 2007, Zelikova and Breed 2008). Despite this evidence, whether ant-mediated dispersal is a generalized or specialized interaction has never been tested rigorously, particularly in a geographical context.

In this study, we investigate the generalization–specialization pattern of the herb *Helleborus foetidus* L. (Ranunculaceae) on its ant dispersers over a considerable part of its geographical range. We follow an approach centered on individual plants. (See a justification for this approach and our quantitative measurement of generalization in *Methods*.) The adoption of such a perspective allows us to test the null hypothesis that ant-mediated dispersal is a generalized mutualism. At least two premises should be confirmed to validate this hypothesis: (1) diaspores are dispersed by multiple ant-visitor species, and (2) diaspore dispersal is significantly equitable among dispersers. Alternatively, the interaction will be specialized if: (1) diaspores are dispersed by one or few ant visitor species, and (2) diaspore dispersal is significantly uneven among dispersers. We examine the extent of the generalization of this plant species on its ant dispersers across different populations on the Iberian Peninsula. We further investigate whether generalization (or alternatively, specialization) is a species-level trait (that is, if in all populations and years a roughly similar level of generalization on ant dispersers is recorded), or on the contrary, whether ant disperser generalization is an attribute of local populations (that is, if ant disperser generalization varies across populations or years). Finally, we examine the consequences of the level of generalization for early seed dispersal by analyzing the covariation between the visitor diversity and the early

dispersal success. We focus here exclusively on the importance that diversity of dispersers has for seed removal, an issue that has been largely ignored in ant–plant mutualism studies (but see Gove et al. 2007). Other putative determinants of seed dispersal success, like diaspore traits or, in particular, the diaspore visitor abundance are not considered in this study, but they have been addressed elsewhere (Rey and Manzaneda 2007, Manzaneda and Rey 2008). Given that the generalization and specialization of animal mutualistic interactors are two sides of the same coin, and the current predominant paradigm is generalization (see the preceding references), we will hereafter use the term “generalization.”

Limitations and measurement of plant ecological generalization on ant dispersers

A rigorous measurement of the plant ecological generalization on animal interactors should be quantified unequivocally from data obtained on individuals plants (for example, from visitor censuses or surveys of plant specimens; Herrera [2005]). A measurement of generalization may be obtained for a particular ecological situation from the total array of individuals sampled, for example, in a population or in different years. It may be also derived at the species level from the total array of individuals sampled across plant populations.

Unlike pollination studies, the use of censuses on individual plants to characterize interactor assemblages in ant–plant interactions is virtually confined to ant-guard mutualisms (e.g., Horvitz and Schemske 1990). In most ant seed dispersal studies, characterization of both the ant disperser assemblage and its interactive behavior has been approached using artificial seed depots presented in “cafeteria” experiments or other similar methods (e.g., Culver and Beattie 1978, Wolff and Debussche 1999, Gove et al. 2007, Zelikova and Breed 2008, but see Kjellsson 1985, Manzaneda et al. 2007), and/or by diaspore offerings to ants (e.g., Garrido et al. 2002). The use of baits (i.e., seed depots) to estimate generalization (or visitor diversity) has, however, biases and problems inherent to its artificial nature. Among these biases are bait monopolization by the dominant species and atypical worker recruitment (see Bestelmeyer et al. 2000), and unrealistic exposure of diaspores that do not take into account true diaspore availability or the ants’ daily foraging pattern (e.g., Wolff and Debussche 1999, Gove et al. 2007, Zelikova and Breed 2008, but see Turnbull and Culver 1983, Boulay et al. 2007a).

Ecological plant generalization on animal mutualistic interactors has been defined frequently in relation to the number of animal taxa involved in the interaction, as distinct from morphological or evolutionary generalization (Waser et al. 1996, Ollerton and Cramer 2002, Herrera 2005). However, species richness alone does not take into account the differential contribution of each animal interactor to plant performance (Sahli and

Conner 2006). Here, we define generalization in terms of the diversity of ant visitors that realized dispersal by removing the diaspores. Different components of diversity have been employed recently to characterize plant generalization on animal mutualistic interactors, such as species richness (Ollerton and Cramer 2002, Herrera 2005) and species evenness (Gove et al. 2007). Though the use of explicit diversity indices (which combine species richness and evenness in a single measure) has recently been suggested to estimate plant generalization (Sahli and Conner 2006), we opt to describe the diversity of ant visitors using both species richness and evenness separately. The reasons for this are basically the same as those that have been argued for abandonment of diversity indices in ecological community studies, i.e., poor biological interpretation, lack of a probabilistic basis, and high sensitivity to the number of species and individuals in the sample (Gotelli and Graves 1996).

Properly estimated species richness constitutes the simplest way to describe diversity (Gotelli and Graves 1996, Gotelli and Colwell 2001). Additionally, it has good discriminant ability (Magurran 1988), which is useful for comparative purposes. In turn, species evenness allows investigators to determine the relative contribution of each ant disperser and to examine whether diaspore visitation is evenly distributed among visitors. Although most evenness indices are not free of problems (Gotelli and Graves 1996) and should not be used alone to measure plant generalization, evenness along with species richness provides an accurate understanding of the generalization pattern in animal-plant mutualisms (Sahli and Conner 2006).

METHODS

Study plant and system

Helleborus foetidus is an evergreen herb distributed throughout central and southern Western Europe (see Plate 1). It is a common species in clearings, patchy scrublands, forest edges, and the understory of deciduous and mixed forests. On the Iberian Peninsula, it grows at middle to high elevations in northern and southeastern mountains. Plants produce inflorescences after several seasons of vegetative growth. Flowers have 1–6 carpels, each of which develops 10–15 elaiosome-bearing seeds. The diaspore (seed plus elaiosome) fresh mass ranges between 5 and 23 mg. The elaiosome, which remains attractive only for a short time (a few hours), is white and soft and comprises ~3–15% of the total diaspore fresh mass. Fruit maturation and seed shedding take place in June–July. Ants are attracted by the lipid-rich elaiosome and remove the diaspore quickly once it has fallen on the ground, or they may climb the plant to collect diaspores directly from dehiscing fruits. The diaspores are carried to the nest, where the elaiosome is removed and eaten, and then the intact seed may be discarded within the nest or on a waste-midden on the

surface. There is no evidence of secondary dispersal from ant nests by other ant species.

On the Iberian Peninsula, nearly 40 ant species interact with *H. foetidus* (Manzaneda et al. 2007). Most of them act as in situ elaiosome consumers (Fig. 1), i.e., nondispersers. However, ant species belonging to the genera *Camponotus* and *Formica*, which behave as legitimate dispersers (i.e., transporting the entire diaspore to the ant nest; Fig. 1), quantitatively dominate the ant-visitor assemblage. (See Appendix A for a complete list of ant taxa, their relative abundance in the study populations, and their interactive behavior.) More information on the dispersal ecology of *H. foetidus* can be found elsewhere (Garrido et al. 2002, Manzaneda et al. 2005, 2007).

Study sites

This study was conducted in June–July (*H. foetidus* seed-shedding period) from 2001 to 2003, in 10 populations at four separate regions (ranging over a distance of ~800 km) on the Iberian Peninsula (Fig. 2; Appendix B). Two of these regions, Cazorla and Mágina, are southeastern mountain systems, which are 100 km apart. The third region was Peña Negra, a central mountain system >400 km north of the Cazorla and Mágina mountains. The last region was Caurel, which is a northwestern mountain system, 750 km from the southern regions and 350 km northwest of the central region of Peña Negra (Fig. 2). These populations cover much of the natural range of *H. foetidus* on the Iberian Peninsula, representing the diverse ecological conditions in which this species occurs. Wide ranges of habitats and ant communities are represented by these populations (Appendix B; see Manzaneda et al. [2007] for details of localities).

Methods and measurement of plant generalization on their ant dispersers

We conducted ant-visitor censuses on individual reproductive *H. foetidus* during the seed release period. In each population, we chose 12–45 reproductive plants and carried out 6–22 ant censuses per plant. Because different ant species often vary in daily foraging activity, censuses were distributed from dawn to dusk according to a random permutation schedule. In each census, we recorded, during a five-minute period, every ant taxon (just workers) visiting dehiscing carpels on the plant or diaspores on the ground below the plant. We also recorded the number of individuals of each ant taxon visiting the plant and the number of diaspores contacted. Specimens of all ant taxa that visited *H. foetidus* seeds during the observation periods were identified by sight and collected at the start of the study for taxonomic determination or corroboration, and thereafter whenever a species was recorded in the censuses for the first time. Most seed visitors were identified to species. Since it was impossible to collect all visitors for corroboration, under- or overestimation of total visitor



FIG. 1. Big formicines such as *Camponotus cruentatus* (left above) or *Camponotus vagus* (left below) are legitimate dispersers of *Helleborus foetidus* diaspores. Small myrmicines such as *Crematogaster sordidula* (right) act as in situ elaisome consumers (nondispersers) or ants with a mixed dispersal behavior. The white appendage in the diaspore is the lipidic-elaisome, which constitutes the attractive part for ants. Photo credits: Victor Parra-Tabla and Antonio J. Manzaneda.

diversity could occur if identification mistakes were common. However, mistakes in the identification of main legitimate dispersers (big-sized formicines) were unlikely given their high recurrence in the censuses and their evident taxonomic features. We conducted a total

of 3535 censuses (17 675 minutes of observation) on 457 reproductive adults, yielding 4972 ant–diaspore interaction records and 2347 cases of diaspore removal. During each census we also noted the interactive behavior of each ant taxon visiting the diaspores, distinguishing

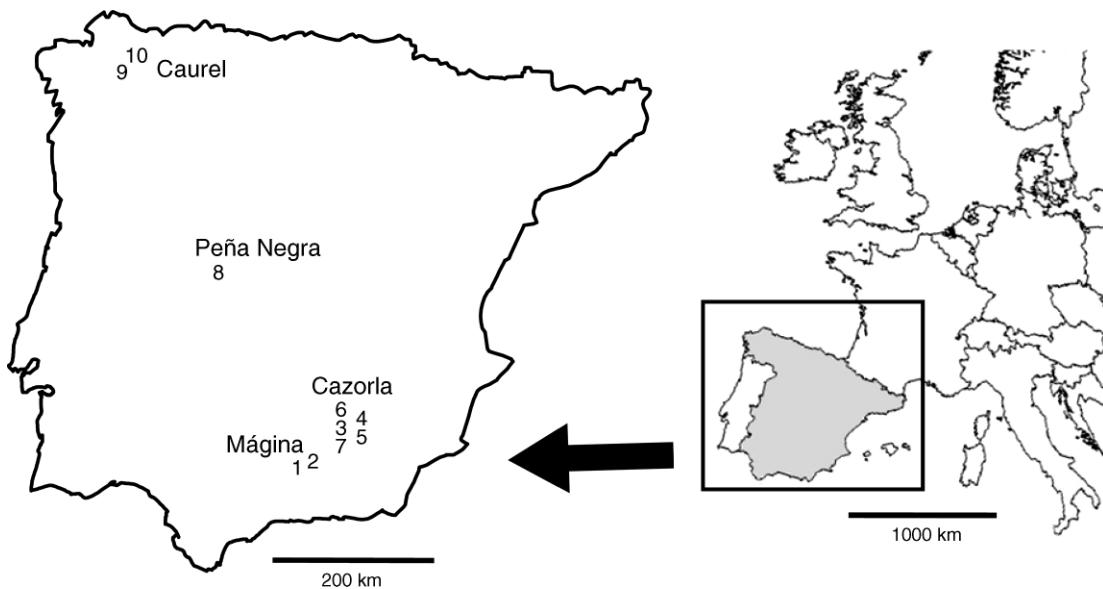


FIG. 2. Map of the Iberian Peninsula (left), showing the location of the 10 study populations. Identification of each population by its number is provided in Appendix B. The two most distant regions (Caurel and Mágina) were ~760 km apart, while the two closest regions (Mágina and Cazorla) were ~85 km apart.

between dispersal and elaiosome consumption. Based on the frequency of these two behaviors, ants were classified into three functional groups. (1) Legitimate dispersers (LD) are ants that behave predominantly as seed removers (i.e., when the percentage of seed removal records was $\geq 75\%$ of its total interactive records). We considered that a seed removal occurred when ants moved the diaspores beyond of the vertical projection of the reproductive stalk on the ground (range 25–90 cm). (2) In situ elaiosome consumers are ants that behave predominantly as elaiosome predators (i.e., when the percentage of in situ elaiosome consumption was $\geq 75\%$ of its total interaction records). (3) Ants with a mixed dispersal behavior (MB) are ants that behave ambiguously (i.e., seed removal and elaiosome consumption records were in similar proportions). *H. foetidus* ant assemblages in these populations have been described in Manzaneda et al. (2007).

In this study, we quantitatively estimated generalization in ant dispersers using the diversity (species richness and evenness, see *Introduction*) of the ant taxa involved in the interaction with *H. foetidus* diaspores, with special attention to LD and MB visitors. To attain a more accurate picture of the plant's perception of visitor species richness, diversity was estimated based on the number of diaspores contacted rather than on the number of ant taxa visiting *H. foetidus* plants (see Herrera 2005). In order to discriminate between incidental visitors and true plant interactors, we also discriminated between the diversity of those ant species that act as true dispersers (LD and MB) and the rest of the ant interactors. Thus, only species richness and evenness of true dispersers were taken into account to estimate plant generalization.

Measurement of the individual seed removal

In each census, the seed removal rate was calculated as the number of removed seeds relative to the instantaneous seed availability (i.e., seeds removed divided by the available number of seeds per plant in the census). The number of available seeds during each census was obtained from summing the total number of seeds on the ground and the number of seeds in dehisced carpels on the plant.

Spatial and temporal variation

Variations among populations in ant visitor species richness, evenness, and its geographic structuring (i.e., the relationship between ant visitor diversity components and geographical parameters) were analyzed for 10 populations (Appendix B) during June and July in 2003. For each population, we chose 26–45 reproductive plants (1993 censuses on 309 plants, in total). To explore interannual variation in ant visitor species richness and evenness, we used data from five populations with more than one year of censuses during the 2001–2003 period. Three populations (Roblehondo, Barranco de la Yedra, and Calvario) were at the Cazorla region, and the others

were at the Peña Negra (Barranco del Toril) and Caurel (Las Cruces-1) regions (Appendix B). At each site and year, we chose 12–45 reproductive plants (2753 censuses on 371 plants, in total).

Data analysis

Individual censuses were the basic sampling units. The species richness was estimated using the Chao2 estimator (Colwell and Coddington 1994). This estimator uses data on rare species collected in the samples to estimate the number of additional species that are expected to be present but were not detected in the samples (Colwell and Coddington 1994). For each Chao2 value, we obtained its log-linear 95% confidence limits using the analytical procedures proposed by Chao (1987). These confidence limits were used to assign statistical significance to the species richness values. In all cases, we computed calculations separately both for total ant visitors and for LD and MB guilds. Species richness values and their log-linear 95% confidence intervals were obtained with EstimateS 7.5 (Colwell 2005).

Generalization of *H. foetidus* on its dispersers was further explored by analysis of species evenness. Species evenness (or equitability) is a component of diversity that permits evaluation of how equal the species abundance is within a particular assemblage (Magurran 1988). We estimated species evenness from two indices: the Hurlbert's probability of an interspecific encounter, PIE (Hurlbert 1971), and dominance. PIE measures the chance that two individuals drawn randomly from the assemblage represent two different species and is calculated as

$$\text{PIE} = \left(\frac{N}{N-1} \right) \left(1 - \sum_{i=1}^s p_i^2 \right)$$

where N is the total number of individuals in the assemblage, s is the number of species in the assemblage, and p_i is the proportion of the entire sample represented by species i . Although the variance of this estimator increases at small sample sizes, PIE remains unbiased even at small sample sizes (Gotelli and Graves 1996). Although this index has the same pitfalls as other evenness estimators (e.g., Pielou's evenness index) in cases of monospecific assemblages, its simple biological and statistical interpretation as a probability (i.e., ranging between 0 and 1) and, more importantly, its independence from abundance makes PIE an ideal estimator of species evenness in the context of our study. This feature of PIE is especially relevant if we wish to compare evenness between different situations (i.e., populations or years) that cannot be addressed using evenness indices based entirely on abundance (e.g., dominance) or indices largely influenced by the effects of abundance or species richness differences (e.g., Pielou's evenness index). Thus, where several taxa were recorded, we computed a single value of PIE for each population or year.

TABLE 1. Ant visitor species richness (number of species) (Chao2 values) within those functional groups able to remove diaspores, legitimate dispersers (LD), and ants with mixed dispersal behavior (MB), across 10 *Helleborus foetidus* populations in the Iberian Peninsula in 2003.

Population	No. diaspores visited		Species richness (no. species)	
	LD	MB†	LD	MB
Matabejid	407	21	3.9 (3.1–15.1)	1 (1–1.1)
Puerto de la Mata	18	10	1 (1–1.29)	2 (2–3.4)
Barranco la Yedra	81	61	3 (3–4.1)	2.8 (2.1–12.5)
Calvario	165	29	2.2 (2–5.5)	1 (1–1.9)
Roblehondo	80	1	5 (5–5.8)	...
Roblehondo-Forest	1	8
Linarejos	90	10	1.8 (1.8–2.2)	1 (1–1.9)
Barranco del Toril	5	11	...	1 (1–2.4)
Las Cruces-1	149	11	1.9 (1.9–2.9)	2.1 (1.5–8)
Las Cruces-2	487	0	1 (1–1.9)	...

Notes: Populations with <10 total diaspores visited were not included. The 95% confidence intervals are shown in parentheses. Ellipses indicate that no data are available.

† Only ant visits that yielded diaspore removal were included.

In addition, species evenness was also estimated from dominance, defined as the proportion of diaspores removed by the most common disperser (genera or species of LD and MB exclusively). Although dominance is built entirely from abundance and should not be used per se to analyze variation in ecological specialization, its use here is well justified. First, unlike PIE or Pielou's evenness, it serves as an intuitive parameter in cases of monospecific assemblages. Second, it is a useful index of resource monopolization (diaspores in this study) by a specific taxonomic group (Gotelli and Graves 1996). Thus, it will complement species richness and PIE to measure the specialization of the interaction, especially in those cases where PIE has no resolution. We computed a single value of dominance for each population or year where several taxa were recorded visiting >10 diaspores.

We compared the observed PIE and dominance to that expected if diaspores were evenly removed by the dispersers of the ant visitor assemblage (i.e., scenario of ecological generalization; see also Gove et al. 2007). For that purpose, we created a null model that assumes, at each level of comparison (population or year), an equal number of diaspore removals by each species of the disperser guild. For each level of comparison, we randomized the null model and computed the PIE (or dominance) index 1000 times in order to establish 95% confidence limits with which to compare the observed PIE (or dominance) index. We also used the 95% confidence limits to compare among populations or years. PIE, dominance, and null model simulations were performed using EcoSim 7.0 (Gotelli and Entsminger 2001).

The possibility for geographic structuring in the *H. foetidus* ant-visitor assemblage was examined through multiple regressions of species richness (log-transformed), PIE, and dominance on altitude, latitude (transformed to a decimal scale), and their interaction. Because our data did not satisfy the assumptions of

classic simple or multiple regression analyses (e.g., small sample size, nonnormal errors, and/or presence of outliers [Rousseeuw and Leroy 1987]), we used robust regression techniques. We fitted robust multiple regression models using MM-estimation of the regression slopes. These regressions were carried out for ant dispersers (LD and MB), both separately and jointly. Similarly, we conducted simple robust regressions to explore the relationship of seed removal to visitor species richness, PIE, and dominance. Regressions were conducted using ROBUSTREG procedure implemented in SAS version 9.1 (SAS Institute 2004). Statistical significance of regressions came from R_n^2 , Robust Wald's linear test (Chen 2002).

RESULTS

Interpopulation variation

A total of 25 ant species were recorded across 10 *H. foetidus* populations studied in 2003, 14 belonging to the subfamily Formicinae (mainly genera *Camponotus*, *Formica*, and *Lasius*), 9 belonging to subfamily Myrmicinae (mainly genera *Aphaenogaster*, *Tetramorium*, *Crematogaster*, and *Pheidole*), and 2 belonging to the subfamily Dolichoderinae (genus *Tapinoma*). Species richness of total ant visitors varied notably among populations (Appendix C), ranging between 2.33 (Las Cruces-2) and 14 (Roblehondo). LD species richness also varied significantly among populations (Table 1). The highest species richness of LD was recorded at Roblehondo, reaching values of 5 (Table 1). In contrast, the richness of LD was minimal in the populations of Las Cruces-1, Linarejos and, especially, Las Cruces-2, where we recorded only one species (*Formica lugubris*; Table 1). Species richness of MB ranged between 1 and 2.8 (Table 1).

In populations visited by more than one disperser species, the observed PIE was in all but one case significantly lower than expected by chance (Fig. 3). In all but one population, the dominance within the

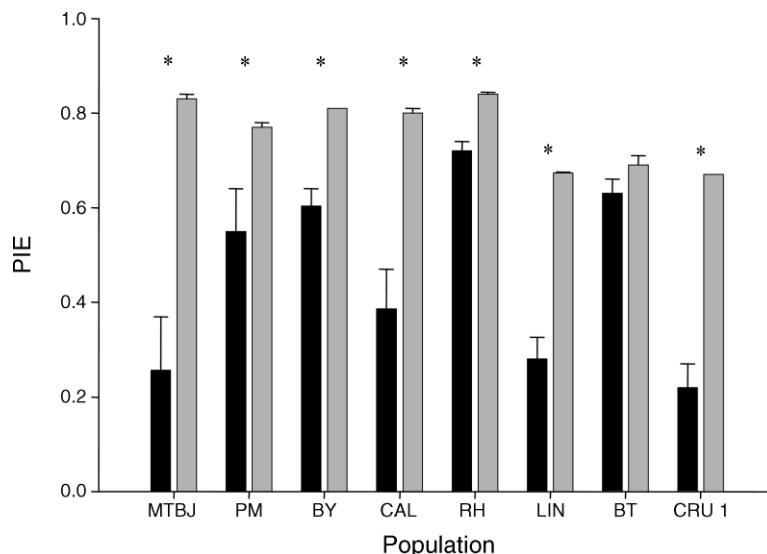


FIG. 3. Observed (black bars) and expected (gray bars) probability of an interspecific encounter (PIE) and 95% confidence upper limit across eight *H. foetidus* populations of the Iberian Peninsula in 2003 that were visited by >1 species of dispersers. An asterisk indicates an observed PIE that was significantly ($P < 0.05$) different from the null model. Population abbreviations are: MTBJ, Matabejid; PM, Puerto de la Mata; RH, Roblehondo; BY, Barranco de la Yedra; CAL, Calvario; LIN, Linarejos; BT, Barranco del Toril; Cru 1, Las Cruces-1.

disperser guild was higher than that expected by chance (Table 2). Across all populations, the disperser guild was significantly dominated by only three genera (*Camponotus*, *Cataglyphis*, and *Formica*; Table 2). In five out of six populations from the two southern regions (Mágina and Cazorla), the disperser guild was significantly dominated by a single genus and species (*Camponotus*, *C. cruentatus*; Table 2). Only where *C. cruentatus* was absent did the disperser guild become dominated by other species; this was the case in Puerto de la Mata, where *Cataglyphis velox* significantly dominated the disperser guild (Table 2). In the northern region (Caurel), the disperser guild was totally monopolized

by a single genus and species (*Formica*, *F. lugubris*; Table 2) in both studied populations. In the central region (Barranco del Toril at Peña Negra), the most active genus, *Lasius*, did not significantly dominate the disperser assemblage; however, at the species level, *L. emarginatus* was significantly dominant (Table 2).

There was no significant relationship between species richness of the total ant-visitor assemblage and latitude, elevation, or their interaction (Appendix D). Similarly, we did not obtain any significant relationship between species richness of true dispersers (LD and MB, both separately and jointly) and geographical parameters (Appendix D). Likewise, observed PIE and dominance

TABLE 2. Dominance analysis of disperser (LD and MB) genera and species across 10 populations from the Iberian Peninsula in 2003.

Population	Genus-level dominance		Species-level dominance	
	Observed†	Dominant genus	Observed†	Dominant species
Matabejid	85.9 (78.7–92.5)	<i>Camponotus</i>	85.9 (78.7–92.5)	<i>C. cruentatus</i>
Puerto de la Mata	62.6 (50–75)	<i>Cataglyphis</i>	62.6 (50–75)	<i>C. velox</i>
Barranco la Yedra	52.8 (45–60)	<i>Camponotus</i>	52.8 (45–60)	<i>C. cruentatus</i>
Calvario	77.3 (71.2–83.7)	<i>Camponotus</i>	77.3 (71.2–83.7)	<i>C. cruentatus</i>
Roblehondo	66.2 (65.3–68)	<i>Camponotus</i>	44.2 (42.7–45.3)	<i>C. cruentatus</i>
Roblehondo-Forest‡
Linarejos	90.9 (88.7–93.7)	<i>Camponotus</i>	83.8 (81.2–87.5)	<i>C. cruentatus</i>
Barranco del Toril	54.7 (50–61.1)	<i>Lasius</i>	54.7 (50–61.1)	<i>L. emarginatus</i>
Las Cruces-1	100§	<i>Formica</i>	87.9 (85–92)	<i>F. lugubris</i>
Las Cruces-2	100	<i>Formica</i>	100	<i>F. lugubris</i>

Notes: Genera or species that are significantly ($P < 0.05$) dominant (see *Methods*) are shown in boldface type. The 95% confidence intervals are shown in parentheses. Ellipses indicate that no data are available.

† Observed dominance values were calculated from the percentage of diaspores removed by the most abundant disperser (genus or species).

‡ Fewer than 10 diaspores removed.

§ Only one genus/species observed; thus no statistical test is possible.

TABLE 3. Ant visitor species richness (Chao2 values) within those functional groups able to remove diaspores, legitimate dispersers (LD), and ants with mixed dispersal behavior (MB), in five *H. foetidus* populations sampled in consecutive years.

Population	Year	No. diaspores visited		Species richness	
		LD	MB†	LD‡	MB‡
Barranco la Yedra	2001	65	4	2 (2–2.1)	...
	2002	87	1	3 (3–3.5)	...
	2003	81	61	3.1 (2.8–7.9)	...
Calvario	2001	168	0	3 (3–3.3)	...
	2002	120	18	3.9 (3.1–15)	1 (1–1.3)
	2003	165	29	2.9 (2.5–8.3)	2.1 (1.5–8)
Roblehondo	2002	118	5	6.4 (6–13.6)	...
	2003	80	1	5 (5–5.8)	...
Barranco del Toril	2002	0	30	...	1 (1–1.1)
	2003	5	11	...	1 (1–1.3)
Las Cruces-1	2002	71	15	2 (2–2.4)	2 (2–4)
	2003	149	11	1.9 (1.9–2.9)	1.5 (1–6.5)

Notes: Populations with <10 total diaspores visited were not included. The 95% confidence intervals are shown in parentheses.

† Only ant visits that yielded diaspore removal were included.

‡ In some years, <10 diaspores were visited; thus, interannual comparison is not possible. Ellipses indicate that no data are available.

were not related to any geographical parameters ($P > 0.05$ in all cases). Thus, neither total visitor species richness nor the species richness, evenness, or dominance of the disperser guild (LD and MB) was geographically structured.

Temporal variation

Overall, the species richness of ant visitors did not differ significantly among successive years within a locality (Appendix E). In fact, species richness differed significantly between two consecutive years in only one out of five populations (Roblehondo-Forest, Appendix E). Species richness for the guild of LD varied significantly among years at Barranco de la Yedra and Roblehondo (Table 3), and did not change significantly for the rest of the populations. In Barranco de la Yedra, species richness of LD was slightly lower in 2001 than in 2003 (2 species vs. 3.13 species) but not than in 2002 (Table 3). In Roblehondo, species richness of LD was higher in 2002 than in 2003 (Table 3). The low number of diaspores removed by MB limited the interannual comparison in this guild, although in those populations where the comparison was possible, species richness of MB was low in all the years studied (Table 3), and only varied significantly between years in one population, Calvario, with a slightly higher value in 2003 than in preceding years.

Within each population observed, PIE remained, overall, invariant between years (Fig. 4), and it was always significantly lower than expected by chance (Fig. 4). Except for Barranco del Toril in 2003, the observed dominance at the genus level within the disperser guild was always higher than that expected by chance (Table 4). At the species level, the observed dominance within this guild was higher than that expected by chance in all years and populations (Table 4). In the three popula-

tions from the southern region of Cazorla, the disperser guild was constantly and significantly dominated by a single genus and species (*Camponotus*, *C. cruentatus*) (Table 4). In the northern population of Las Cruces-1, the disperser guild was constantly monopolized by a single genus and species (*Formica*, *F. lugubris*, Table 4). In the central region (Barranco del Toril at Peña Negra), observed dominance varied at the genus level between consecutive years (Table 4). In this region, the most active genus, *Lasius*, significantly dominated the disperser assemblage in 2002 but not in 2003 (Table 4). At the species level, *L. emarginatus* dominated the disperser guild in both years (Table 4).

Species richness, evenness and seed removal rate

Seed removal varied significantly among populations (see Manzaneda and Rey 2008, for details on these results), but there was no significant relationship between species richness and seed removal ($R_{1,10}^2 = 0.10$, $\chi^2 = 0.10$, $P = 0.75$). Species richness of LD and MB was not related to seed removal (LD, $R_{1,7}^2 = 0.02$, $\chi^2 = 0.02$, $P = 0.88$, MB, $R_{1,19}^2 = 0.12$, $\chi^2 = 0.12$, $P = 0.73$). Likewise, the species richness of the disperser guild (LD and MB jointly) was unrelated to seed removal ($R_{1,10}^2 = 1.89$, $\chi^2 = 1.89$, $P = 0.17$). This lack of relation was also found for observed PIE, dominance, and seed removal ($P > 0.05$ in all cases). Thus, early dispersal success at the interpopulational scale was independent of variation in the components of diversity of the ant visitor assemblages.

DISCUSSION

During the study period, a total of 37 ant species interacted with *H. foetidus* diaspores. At first glance, this high number of species would suggest that *H. foetidus* is a generalist with respect to its interaction with

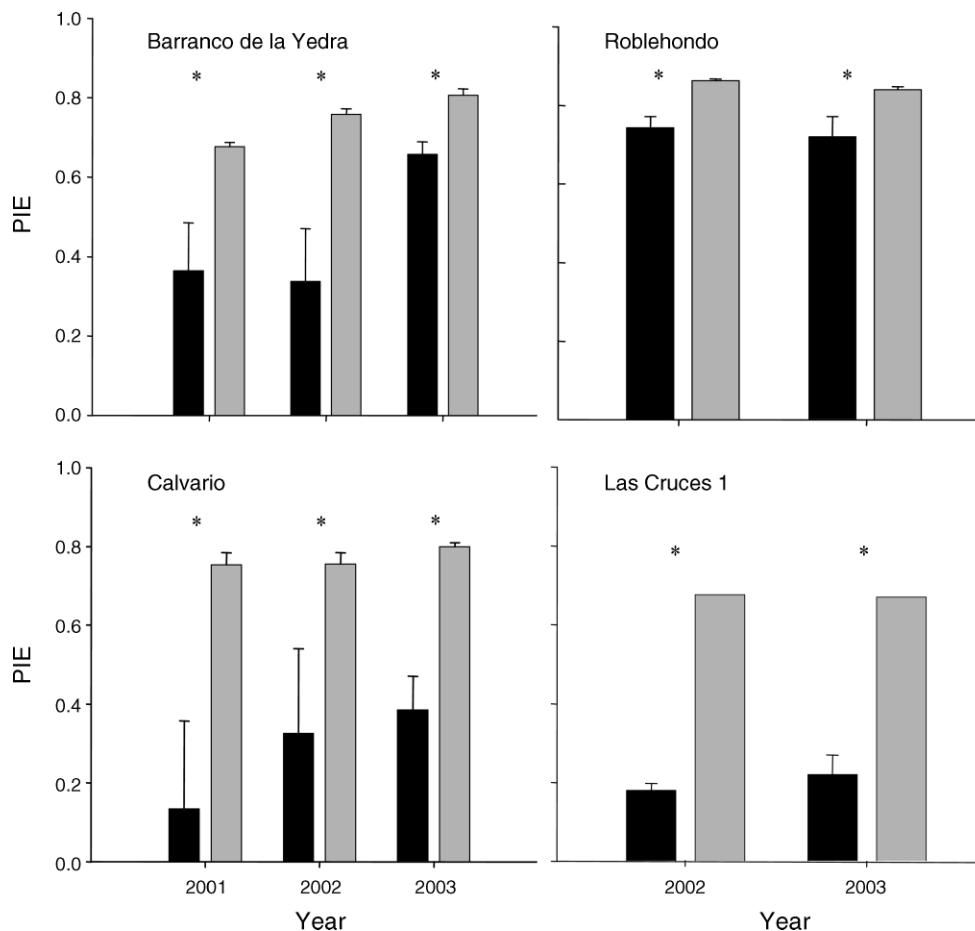


FIG. 4. Observed (black bars) and expected (gray bars) probability of an interspecific encounter (PIE) and 95% confidence upper limit across four *H. foetidus* populations of the Iberian Peninsula that were visited by >1 species of dispersers in successive years. Asterisks indicate observed PIE's that were significantly ($P < 0.05$) different from the null model.

ant seed dispersers, which is consistent with the view that ant–seed dispersal mutualisms are generalized interactions (Beattie and Hughes 2002). However, the adoption of an individual plant's perception of ant

disperser diversity provides new insight into the nature of specialization–generalization in ant–seed dispersal mutualisms. First, a closer look at the *H. foetidus* dispersal shows that only one or a few ant species

TABLE 4. Dominance analysis of disperser (LD and MB) genera and species over five *H. foetidus* populations in the Iberian Peninsula sampled in consecutive years.

Population	Year	Genus-level dominance		Species-level dominance	
		Observed	Dominant genus	Observed	Dominant species
Barranco la Yedra	2001	78.1 (68.7–87.5)	<i>Camponotus</i>	78.1 (68.7–87.5)	<i>C. cruentatus</i>
	2002	80.6 (75–86.7)	<i>Camponotus</i>	79.5 (68.7–90.6)	<i>C. cruentatus</i>
	2003	53.1 (40.6–68.7)	<i>Camponotus</i>	53.1 (40.6–68.7)	<i>C. cruentatus</i>
Calvario	2001	92.9 (80–100)	<i>Camponotus</i>	92.9 (80–100)	<i>C. cruentatus</i>
	2002	81.1 (65–95)	<i>Camponotus</i>	81.1 (65–95)	<i>C. cruentatus</i>
	2003	77.3 (71.2–83.7)	<i>Camponotus</i>	77.3 (71.2–83.7)	<i>C. cruentatus</i>
Roblehondo	2002	62.2 (45–80)	<i>Camponotus</i>	37.4 (30.2–44.7)	<i>C. cruentatus</i>
	2003	66.2 (65.3–68)	<i>Camponotus</i>	44.2 (42.7.45–3)	<i>C. cruentatus</i>
Barranco del Toril	2002	100†	<i>Lasius</i>	100	<i>L. emarginatus</i>
	2003	54.7 (50–61.1)	<i>Lasius</i>	54.7 (50–61.1)	<i>L. emarginatus</i>
Las Cruces-1	2002	98.6 (98.4–100)	<i>Formica</i>	90 (89.2–92.3)	<i>F. lugubris</i>
	2003	100†	<i>Formica</i>	87.9 (85–92)	<i>F. lugubris</i>

Notes: Genera or species that are significantly ($P < 0.05$) dominant (see *Methods*) are shown in boldface type. The 95% confidence intervals are shown in parentheses.

† Only one genus/species was observed; thus no statistical test was possible.

accounted for realized seed removal. Only two LD species, *C. cruentatus* in the south and *F. lugubris* in the north, were responsible for the vast majority of visits resulting in transportation of diaspores. The two assertions needed to verify ecological specialization in ant-mediated dispersal (see *Introduction*) were thus confirmed, suggesting that ant–seed dispersal in *H. foetidus* is specialized. Second, its consistency over space and time suggests that such a specialization is a species-level trait rather than a mere attribute of local populations where *H. foetidus* grows. Finally, the variation in diversity components of total ant visitors and true dispersers did not explain the variation in seed removal, suggesting that ant species diversity is not a determinant of early seed dispersal success. Our results thus contradict suggestions that plant–ant disperser relationships are highly diffuse (but see Gove et al. 2007), and shows that only a very small fraction of visitors are important in seed dispersal.

Interpopulational variation in ant disperser diversity

Among populations, the ant disperser assemblage of *H. foetidus* was composed of a great number of ant species (25 taxa were recorded in 2003 across our 10 study populations); however, the number of species recorded per population was considerably lower, and differed notably among populations. Although some of the southern populations, such as Roblehondo or Barranco de la Yedra, reached high values of total species richness, ant visitor diversity (both species richness and evenness) did not show any overall geographic pattern. Similarly, we did not find any latitudinal or elevational trend in the diversity of true dispersers (LD alone or jointly with MB), though again some southern populations (Roblehondo, Barranco de la Yedra, or Matabejíd) showed higher species richness than northern populations, which tended to be more monospecific. The lack of geographic gradients here contravenes the results of studies showing latitudinal and altitudinal gradients, at different scales, in ant species diversity (Gotelli and Ellison 2002, Kaspari et al. 2003, Sanders et al. 2003, but see Gove et al. 2007). Lack of such gradients are expected when the explored latitudinal or elevational ranges are relatively small (as in this study), because local or regional effects may mask diversity gradients (Gotelli and Ellison 2002).

Both species richness and the evenness of ant dispersers varied among populations, although the distribution of the diaspore visits among ant species was consistently lower than expected by chance (indicating visit overdominance by a few ant species) in most of the studied populations. In fact, only one or very few ant genera and species were actually responsible for the diaspore removals. Specifically, large formicines, such as *C. cruentatus* or *F. lugubris*, removed the vast majority of *H. foetidus* diaspores. Only where these species were absent did other species dominate the visits to the

diaspores. This was the case at Puerto de la Mata (Mágina region) and at Barranco del Toril (Peña Negra region), where the LD large formicine *Cataglyphis velox* and the MB medium-sized formicine *Lasius emarginatus* became dominant. However, the absolute number of diaspores that these species removed where dominant was rather low (18 and 11, respectively, Table 1), and much lower than the number of seeds removed by *C. cruentatus* and *F. lugubris* in their respective populations (Table 1). Taken together, our results strongly demonstrate that, despite the “open” nature of the diaspore reward, *H. foetidus* greatly relies on the presence of *C. cruentatus* or *F. lugubris* at the population level for seed dispersal. Therefore, the mutualistic interaction between *H. foetidus* and its ant dispersers is ecologically specialized at this spatial scale. Two very recent studies have also recorded a disproportionate importance of a few ant species for seed dispersal at the interpopulational scale, one through a rainfall gradient in western Australia (Gove et al 2007), and the other through a disturbance gradient in a tropical dry forest of Costa Rica (Zelikova and Breed 2008). These results, together with recent findings from the analysis of community-level mutualistic networks in ant-guard systems, which confirmed asymmetrical specialization in several communities (Guimarães et al. 2006), suggest that ecological specialization may be more frequent in ant–plant mutualisms than initially thought.

Interestingly, the two primary dispersers of *H. foetidus* were themselves geographically structured (*C. cruentatus* in southern populations and *F. lugubris* in the north), which could have potential consequences for the evolution of this mutualism. For example, *C. cruentatus* and *F. lugubris* may exert contrasting selective pressures on plants, leading to geographic differentiation in ecologically important traits related to dispersal. Both species have been shown to respond similarly to dispersal-related plant traits of *H. foetidus*, such as seed and elaiosome size, diaspore release timing, and amount of oleic acid contained in the elaiosome (Boulay et al. 2006, 2007b). As a consequence, a differential selection of these traits is not expected. However, differentiation may still arise if the final ecological outcome of the interaction is not equivalent between the two ant species. Interestingly, these two ant species show differences in some of the qualitative components of their dispersal efficiency. Survival of seeds and seedlings is significantly higher in nests of *C. cruentatus* than in nests of *F. lugubris* (Manzaneda 2005, Manzaneda et al. 2005). To date, very few studies have compared the selection of dispersal-related plant traits between ant disperser species or assemblages (but see Alcántara et al. 2007), and none did it through considering quantitative and qualitative components of dispersal on individuals plants (Giladi 2006). Future work on *H. foetidus* dispersal should investigate whether observed natural variation in dispersal-related plant traits (Garrido et al. 2002, Rey and Manzaneda 2007) arises from variations

in the dispersal service provided by these two primary dispersers.

Interannual variation in the extent of specialization

Within the same population, there was little between-years variation in species diversity. Despite slight variations among successive years in the number of ant disperser species in some populations, the identity of the ant dispersers was the same during all study periods. In the three populations in Cazorla, *C. cruentatus* removed the most diaspores each year. Similarly, *F. lugubris* and *L. emarginatus* also monopolized the majority of the diaspore removals in their respective populations during consecutive years. This suggests a temporally predictable species interaction that may involve consistent selective pressures on plant dispersal traits.

Temporal fluctuations in relative abundance, species composition, and size of the animal interactive assemblages of plants have been pointed out as fundamental ecological factors promoting ecological generalization in animal-plant mutualisms (Gómez 2002). This is because such fluctuations would lead to unpredictable shifts in the direction and magnitude of the selective pressures exerted on plants (Herrera 1988, Horvitz and Schemske 1990, Gómez 2002). Compared to the common large fluctuations in abundance and assemblage size described for other seed dispersers (e.g., birds [Herrera 2002]), ant nests are temporally more stable, which might explain the lack of fluctuation in the major dispersers detected here. Although a longer temporal series is needed, the temporal congruence described here reasserts that the relationship between *H. foetidus* and its ant dispersers is specialized.

Ant visitor diversity and seed dispersal success

Our results show an absence of relationship between plant visitor diversity and individual seed dispersal success. Moreover, this lack of relationship was confirmed when considering diversity of the LD alone or jointly with MB. Our results clearly suggest that ant species diversity is not a determinant of early dispersal success in *H. foetidus*, which is rather determined by the presence or absence of a particular ant species. In other words, seed removal is a function of ant species identity (see also Gove et al. 2007, Zelikova and Breed 2008). This does not neglect, however, the importance of others factors (such as the local abundance of LD or some plant traits) influencing early dispersal success, as has been recently shown in our study system (e.g., Boulay et al. 2007b, Rey and Manzaneda 2007, Manzaneda and Rey 2008).

Toward a new scenario in the generalization–specialization of ant–seed dispersal mutualisms

In contrast to the spatial and temporal variation in size and specific composition of the ant disperser assemblages described in most myrmecochory studies (e.g., Pudlo et al. 1980, Wolff and Debussche 1999,



PLATE 1. Inflorescence of *Helleborus foetidus*. Fruit maturation and seed shedding take place in June–July. Ants may gather the diaspores from the ground once they are released or collect them directly from the dehiscing carpels. Photo credits: A. J. Manzaneda.

Garrido et al. 2002, but see Gove et al. 2007), the interaction between *H. foetidus* and its ant dispersers was specialized at the scales analyzed here. This suggests that specialization of this plant with respect to its ant dispersers was reliant on plant species traits rather than on population particularities, e.g., local impoverishments of the LD guild.

The analysis of which plant traits determine such ecological specialization is beyond the scope of this study. Nonetheless, it could be argued that diaspore size relative to ant size, seasonal and daily timing of diaspore release in relation to activity pattern of ant dispersers, as well as the attractiveness of the elaiosome, will presumably play a major role (Turnbull and Culver 1983, Hanzawa et al. 1985, Oberrath and Böhning-Gaese 2002, Boulay et al. 2006, 2007a, b; but see Ruhren and Dudash 1996). Should these plant traits determine ant-mediated dispersal specialization, then some predictions of species variation in specialization can be suggested. For example, it is expected that the smaller the diaspore of a particular myrmecochorous plant, the more generalized it will be with

respect to its ant dispersers, because the range of ant species behaving as LD will be wider. In contrast, the bigger its diaspores, the more specialized it will be because the range of ant species acting as LD will be limited. Similarly, plants with dried long-lived elaiosomes (which remain attractive for a long time) and a long diaspore release period (typically, sclerophyllous shrubs in the Southern Hemisphere [Beattie and Hughes 2002]) will be less specialized in their dispersers than plants with fleshy and soft elaiosomes (which remain attractive only for a few hours) and restricted diaspore release periods (typically, herbs in temperate forests in the northern hemisphere [Beattie and Hughes 2002]). Given the geographical division in the nature of the elaiosome (Beattie and Hughes 2002), geographical variation (between the two hemispheres) in specialization may exist, with myrmecochorous plants of the northern hemisphere showing higher specialization than those in the south. A rigorous verification of these predictions in future studies will require, in any case, the adoption of an approach centered on individual plants and the use of truly comparable estimates of ant disperser diversity.

In short, although our data are observational, this paper has particular merit because it analyzes the extent of generalization of an ant dispersal system over a broad geographic region and in several years, and because it examines ant–seed interactions at natural seed-fall events. Together with the recent findings of other studies, it suggests that the view of myrmecochory as a predominantly generalized interaction should be reevaluated. We advocate an alternative scenario to extensive generalization, where generalization is replaced by ecological specialization when intrinsic traits of the plant, rather than the ecological context in which the interaction takes place, determine dispersal success.

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APPENDIX A

Ant taxa recorded on reproductive plants of *H. foetidus* (*Ecological Archives* E090-216-A1).

APPENDIX B

Locality names, geographical coordinates, elevations, and short description of study sites (*Ecological Archives* E090-216-A2).

APPENDIX C

Ant visitor species richness (Chao2) for 10 *H. foetidus* populations in the Iberian Peninsula in 2003 (*Ecological Archives* E090-216-A3).

APPENDIX D

Robust multiple linear regressions examining the relationship among ant species richness and geographical parameters (*Ecological Archives* E090-216-A4).

APPENDIX E

Ant visitor species richness (Chao2) across five *H. foetidus* populations in the Iberian Peninsula in consecutive years (*Ecological Archives* E090-216-A5).