Geographic and temporal variation in the ant–seed dispersal assemblage of the perennial herb *Helleborus foetidus* L. (Ranunculaceae)

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Spatio-temporal variations in the composition of the animal interactive assemblages may result in variations in selective pressures on the plants. In ant–seed dispersal mutualisms, the study of the magnitude of spatial and temporal variation of ant assemblages is rarely studied, limiting inferences and generalizations on the evolution of this mutualism. Here, we describe the ant–disperser assemblage of the myrmecochorous herb *Helleborus foetidus* in 14 populations across the Iberian Peninsula, and dissect the variation in the assemblage into spatial and temporal components as a first step to evaluate the evolutionary potential of this interaction. The ant–visitor assemblage of *H. foetidus* was mainly represented by species of Formicinae and it was highly diverse and variable in composition and function. Ants behaving as legitimate dispersers and those with mixed behaviour numerically dominated the assemblage compared with elaiosome consumers. The magnitude of the spatial variation was higher than the temporal variation, suggesting that the relative frequency of each functional group will be more foreseeable among years in each population than among populations. At the expense of further analysis of the effects of such variation on dispersal success, we can envisage a selection mosaic scenario, where local adaptive responses of plants might arise as a result of local variations in the specific composition and function of the assemblage. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **92**, 135–150.

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INTRODUCTION

Variation in space and time in the animal assemblage interacting with a plant species is a major determinant of the lack of prediction often found in the selection pressures experienced by plants (Herrera, 1988). Inconsistency in size, species composition, or interactive behaviour of the animal assemblage may limit the evolutionary potential of the plant–animal interactions. This is because one prerequisite for reciprocal evolution to occur in plant–animal interactions is some degree of predictability in mutual selective pressures (Janzen, 1980; Horvitz & Schemske, 1984; Herrera, 1985; Thompson & Cunningham, 2002; Strauss, Sahli & Conner, 2005). If the diverse animal species that form the interactive assemblage have differential effects on plant fitness, then spatio-temporal variations in the composition of the assemblage will result in variations in selective pressures on the plant (Herrera, 1988; Thompson, 2005; but see Zamora, 2000). In plant–animal mutualisms, the study of the magnitude of spatial and temporal variation is crucial to understanding their evolution. Since the 1980s, numerous works explored the spatio-temporal variation in the assemblages in plant–animal mutualistic systems (pollination: Herrera,

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1988; Gómez & Zamora, 1999; avian frugivory: Jordano, 1994; Jordano & Schupp, 2000; ant–plant mutualisms: Horvitz & Schemske, 1984, 1990; Hughes & Westoby, 1990; Alonso, 1998; Rico-Gray *et al*., 2004; Rudgers & Gardener, 2004).

One procedure to explore the evolutionary potential of a particular plant–animal mutualism is to dissect it into its quantitative and qualitative components (*sensu* Herrera, 1989), and then examine variations in both components between mutualists. In seed dispersal mutualisms, differences in the quantitative component may arise from variations in abundance of dispersers and rates of plant visitation or from shifts in the feeding behaviour of the dispersers (Jordano & Schupp, 2000).

In the case of myrmecochory (seed dispersal by ants), a number of studies have described important spatio-temporal variations in composition, visitation rates, and dispersal behaviour of the visitor assemblage (Pudlo, Beattie & Culver, 1980; Horvitz & Schemske, 1986; Hughes & Westoby, 1990; Gorb & Gorb, 1999; Garrido *et al*., 2002). These and others studies have noted the important effects of such variations on successive stages of the dispersal cycle, such as seed removal, dispersal distance, seed predation, seed germination, and/or seedling survival (Horvitz & Schemske, 1994; Gómez, Pons & Bas, 2003; Cuautle, Rico-Gray & Díaz-Castelazo, 2005; Manzaneda, Fedriani & Rey, 2005). Consequently, we have improved significantly our knowledge about ant disperser guilds and the ecological consequences from shifts in such guilds, but two important aspects still remain unknown. First, most of these studies were conducted on local frameworks (i.e. the spatial variation in disperser assemblage was examined only in one or few habitats within one or few localities; but see Garrido *et al*., 2002). This narrow look clearly limits inferences and generalizations on the evolution of this mutualism (Beattie & Hughes, 2002), as it does for any other interaction, and broad multipopulational approaches are thus demanded (Cushman *et al*., 1998; Beattie & Hughes, 2002; Thompson, 2005). Second, the bulk of studies on ant–disperser assemblages have ignored variation in time (but see Hughes & Westoby, 1990; Ohkawara, Ohara & Higashi, 1997). However, significant temporal variation, both in size and species composition, is common in ant communities (Herbers, 1989; Retana & Cerdá, 2000). Moreover, it is known that the consequences of spatial and temporal variation for evolution of mutualisms are distinct. Although temporarily stable spatial variation might result in local evolutionary specialization, temporal variation may lead to diffuse selection, particularly if such variation is stochastic (Horvitz & Schemske, 1990).

In the present study, we describe the ant–disperser assemblage of the myrmecochorous herb *Helleborus foetidus* L. (Ranunculaceae) in multiple populations across the Iberian Peninsula during successive dispersal events, and dissect the variation in the disperser assemblage into spatial and temporal components as a first step to evaluate the evolutionary potential of this interaction. We used an approach based on individual plant censuses to analyse the variation in ant assemblage at plant, population, and regional levels and its temporal consistency.

MATERIAL AND METHODS

STUDY SYSTEM AND SITES

Helleborus foetidus is an evergreen herb distributed throughout central and southern of Western Europe (Werner & Ebel, 1994). It is a common species in patchy scrublands, forest edges, and the understory of deciduous and mixed woodlands. In the Iberian Peninsula, it grows at middle to high elevations in northern and south-eastern montane areas. Plants have one or several ramets, each of which produces a single terminal inflorescence after several seasons of vegetative growth. Flowers have one to six carpels, each of which develops 10–16 elaiosome-bearing seeds. Diaspore (seed plus elaiosome) fresh mass is in the range 5–23 mg (Garrido, Rey & Herrera, 2005), and seed mass significantly varies between plants, populations and regions (Garrido *et al*., 2002, 2005). The elaiosome is white and soft, and comprises approximately 3–15% of the diaspore fresh mass. Seed shedding takes place in June and July. Ants are attracted by the elaiosome. 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.

This study was conducted in June and July from 2001 to 2003, at 14 populations (distance between sites in the range 1–760 km; Fig. 1) in four separate regions in the Iberian Peninsula (Fig. 1; Table 1). These populations cover much of the natural range of *H. foetidus* in the Iberian Peninsula, representing the diverse ecological conditions in which this species occurs (Herrera *et al*., 2001). A wide range of habitats and of ant communities are represented through these populations.

CHARACTERIZATION OF THE ANT–DISPERSER ASSEMBLAGE AND ANT INTERACTIVE BEHAVIOUR

To describe the ant–disperser assemblage of *H. foetidus* and to characterize the interactive behaviour of the ants, we grouped together data from the 14 populations along the 3 years of study. In each population,
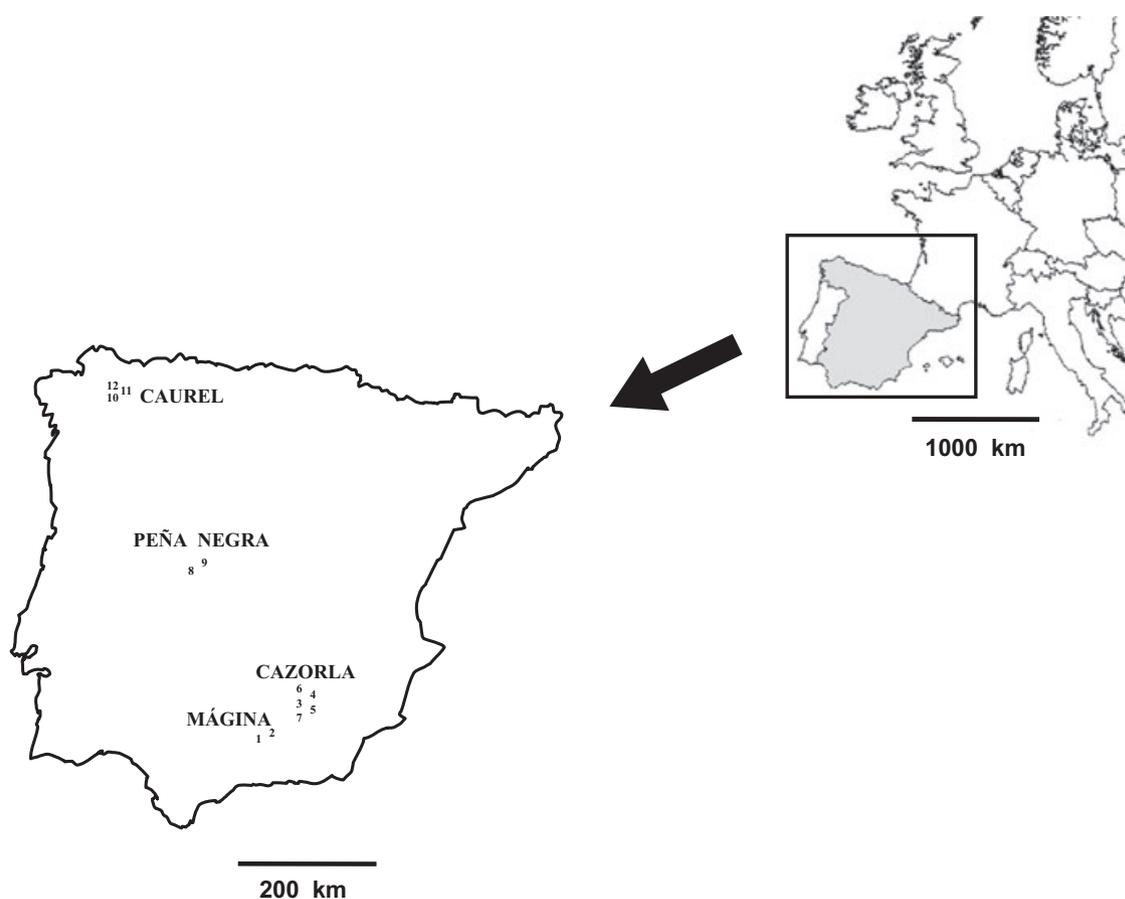
Figure 1. Map of the Iberian Peninsula (left), showing the location of 14 study populations. Identification of each population by its number is provided in Table 1. The two most distant regions (Caurel and Mágina) were approximately 760 km apart, whereas the two closest regions (Mágina and Cazorla) were only approximately 85 km apart.

Table 1. Locations and short description of study sites

Region	Population	Elevation (a.s.l) (m)	Geographical coordiantes	Habitat type
Mágina	Matabejid (1)*	1100	37°43′N, 3°30′W	Mediterranean open scrubland
	Puerto de la Mata (2)*	1650	37°44′N, 3°28′W	Mediterranean open scrubland
Cazorla	Barranco la Yedra (3)*†‡	1200	37°58′N, 3°54′W	*Pinus* and *Quercus* mixed forest
	Calvario (4)*†‡	1450	37°58′N, 3°53′W	Mediterranean open scrubland
	Roblehondo (5)*‡	1270	37°56′N, 3°52′W	Open *Pinus* and *Quercus* mixed forest
	Roblehondo-Forest (6)*‡	1290	37°56′N, 3°52′W	*Pinus* and *Quercus* mixed forest
	Linarejos (7)*	1110	37°56′N, 3°55′W	*Pinus* and *Quercus* mixed forest
Peña Negra	Barranco del Toril (8)*‡	1430	40°26′N, 5°18′W	*Quercus pyrenaica* forest
	Prado de la Francesa (9)	1440	40°27′N, 5°18′W	*Quercus pyrenaica* forest
Caurel	Mostad (10)	1200	42°39′N, 7°7′W	*Castanea sativa* forest
	Entretaros (11)	1150	42°39′N, 7°7′W	Open scrubland pasture and ferns
	Las Cruces-Wood (12)	1500	42°36′N, 7°6′W	*Pinus sylvestris* plantation
	Las Cruces 1 (13)*‡	1500	42°36′N, 7°6′W	Open scrubland pasture and ferns
	Las Cruces 2 (14)*	1500	42°36′N, 7°6′W	Open scrubland pasture and ferns

Symbols denote populations used to explore spatial (*), temporal (†), or spatio-temporal (‡) variations in the ant–disperser assemblage. Numbers in parentheses indicate the location of the population, shown in Figure 1.

Table 2. Sampling effort in each population and year

Population	2001	2002	2003	Total
Matabejid (30)	–	–	366/1830	366/1830
Puerto de la Mata (29)	–	–	143/715	143/715
Barranco la Yedra (12,24,26)	218/1090	161/805	143/715	522/2610
Calvario (12,22,28)	178/890	181/905	146/730	505/2525
Roblehondo (26,34)	–	208/1040	209/1045	417/2085
Roblehondo-Forest (24,29)	–	158/790	149/745	307/1535
Linarejos (26)	–	–	148/740	148/740
Barranco del Toril (29,30)	–	257/1285	141/705	398/1990
Prado de la Francesa (30)	–	309/1545	–	309/1545
Mostad (10)	105/525	–	–	105/525
Entretaros (10)	100/500	–	–	100/500
Las Cruces-Wood (30)	–	300/1500	–	300/1500
Las Cruces 1 (30,45)	–	270/1350	334/1670	604/3020
Las Cruces 2 (30)	–	–	214/1070	214/1070

The number of plants censused in each year is shown in parenthesis. Numbers separated by a solidus denote the total number of censuses at each population and time of observation (min), respectively.

we chose between ten and 45 adult plants of *H. foetidus* (Table 2). We conducted between 6–22 censuses for each adult plant (Table 2). Censuses in each population were distributed through 2–3 weeks during the seed release period of the species (June and July). In each census, we observed and recorded, during a 5-min period, every ant taxon (just workers) seen visiting *H. foetidus* deshicent carpels on the plant or the diaspores on the ground below the plant. In addition, in each census, we also recorded the number of individuals of each ant taxon visiting the plant, and the number of seeds contacted. We typified two kinds of visits to the plant: exploratory (ants just 'antennate' or examine carpels or seeds, but this does not result in either seed removal or elaiosome consumption; pick-up attempts are included in this type of behaviour) and interactive visits (those yielding in seed removal or *in situ* elaiosome consumption).

Because different ant species often show different daily activity to forage (Cerdá, Retana & Manzaneda, 1998), which could affect the accurate characterization of ant–disperser assemblage, in each population we censused every individual plant from 09.00 h to 22.00 h GMT (with an even effort between hours), according to a random permutation scheme. Although, in some populations, ant species exist with night-time habits (e.g. *Camponotus sylvaticus* or *Camponotus pilicornis*), sporadic nocturnal observations on plants dismissed any foraging activity of these ants on *H. foetidus*. The total sampling effort in this study was 4438 censuses (22 190 min) in 523 reproductive adults (Table 2).

To determine the relative importance of each ant species within the ant–disperser assemblage, we used three different parameters based exclusively on interactive visits per plant: the mean relative frequency of visits made by each ant taxon, the mean proportion of seeds removed by each taxon, and the proportion of plants within a population visited by each taxon. To characterize the interactive behaviour for each ant species, we used the proportion of visits that results in seed-removal or elaiosome-predation out of the total interactive events for a given ant species, calculated from the total number of records from all years and all study populations. Then, every ant species was assigned to one of three functional groups of interactive behaviour: (1) legitimate disperser, an ant which behaves predominantly as seed remover (i.e. when the percentage of seed removal records was $\geq 75\%$ of its total interactive records); (2) elaiosome consumer, an ant which behaves predominantly as elaiosome predator (i.e. when the percentage of *in situ* elaiosome consumption was $\geq 75\%$ of its total interactive records); observations confirmed that these species almost invariably consumed the whole elaiosome of the diaspore; and (3) mixed behaviour ('mixed' hereafter), an ant which behaves indistinctly (i.e. seed removal and elaiosome consumptions records were similarly represented). To characterize the function of the ant–visitor assemblage (i.e. the relative frequency of each interactive behaviour) in each population, we took into account how each ant species was behaviourally categorized and its relative abundance in the ant assemblage.

SPATIAL AND TEMPORAL VARIATION

Inter-regional and interpopulational variation in ant–disperser assemblage was examined at ten populations during June and July in 2003 (Table 1; Fig. 1). We chose 26–45 reproductive plants per population (Table 2). We conducted 1993 censuses on 309 plants (Table 2).

Temporal variation in the ant–disperser assemblage was examined over a 3-year period (2001–03) in Barranco de la Yedra and Calvario, both located in Cazorla (Table 1; Fig. 1). In this case, we chose between 12 and 26 plants in each population (Table 2). We conducted 1027 censuses on 126 reproductive plants.

The spatio-temporal pattern of variation in the ant–disperser assemblage was examined across three scales of variation (region, population, and year). We employed exclusively data from populations with more than 1 year of censuses (six populations from three regions; Table 1, Fig. 1). We used 2753 censuses from 371 reproductive adults.

In all these analyses, we determined, for each population and year, the species composition and the relative importance of each ant species in the ant–visitor assemblage as described above. Furthermore, in each population, we determined the relative frequency of each interactive behaviour.

DATA ANALYSIS

The interactive behaviour of the ants (i.e. the proportion of a particular behaviour relative to the total interactive records) was always modelled with binomial error. We fitted generalized linear models (when all factors in the models were fixed) using the SAS GENMOD procedure (Littell *et al.*, 1996) (SAS Institute Inc.), or generalized linear mixed models (with both fixed and random factors, Schabenberger & Pierce, 2002) using the SAS GLIMMIX macro (Littell *et al.*, 1996) with binomial distribution and logit link function. Ant variation in interactive behaviour was tested considering ant species as fixed factor. In addition, for those ant species present in more than one population or region, we explored whether their behaviour varied between populations or regions (region as fixed factor and population nested within region as random factor).

To characterize the spatial (among populations and regions) and temporal variation in disperser assemblage, we used the proportional similarity Renkonen index, PS:

$$\sum_{I=1}^{n} \min(p_{ai}, p_{bi})$$

where, for n species, p_{ai} is the relative abundance of species i in a population or year a, and p_{bi} is the relative abundance of species i in a population or year b. The PS index ranges from 0 (denoting maximum dissimilarity, i.e. no common taxa between a and b) to 1 (indicating maximum resemblance, i.e. identical taxonomic composition between a and b). We tested the null hypothesis of 'dissimilar assemblage composition' (PS = 0), and assessed the statistical significance for each coefficient of similarity by determination of the 95% confidence limits with a bootstrap procedure (Manly, 1991), using Monte Carlo routines implemented in the POPTOOLS ADD IN 2.6.2 macro (CSIRO; http://www.cse.csiro.au/poptools) for Excel (Microsoft Corp.). Observed PS values were compared to a distribution of randomly generated PS values, calculated for each of 10000 paired draws with replacement from the samples being compared (Jordano, 1994). In addition, we explored whether the similarity of the ant assemblages depended on geographical distance. We built two resemblance matrices, one of them from pairwise dissimilarity coefficients (1 − PS) between populations, and the other one including the pairwise geographical distance between populations. The distance dependence in the similarity of the assemblages was tested using Mantel's test (Manly, 1991).

Regional and interpopulational variation in the relative frequency of the different interactive groups was explored by fitting generalized linear mixed models (region as fixed factor and population nested within region as random factor). We conducted a separate analysis for each functional group. Variation between successive years in relative frequencies was further analysed (year as fixed factor) with a generalized linear model.

Finally, to quantify the relative magnitude of variation (between regions, populations and years) of each interactive behaviour, we performed a variance partitioning analysis (COVTEST statement in the MIXED procedure of SAS) through a hierarchical design with region, population nested within region, and year nested within region and population as effects. The model was fitted with restricted maximum likelihood to take into account the unbalanced nature of this design.

RESULTS

THE ANT–DISPERSER ASSEMBLAGE AND ITS INTERACTIVE BEHAVIOUR

After 3 years of study among the 14 study populations, we recorded a total of 8142 specimens of insects visiting *H. foetidus* diaspores. Of these, 97.5% were ants (Hymenoptera, Formicidae) belonging to 35 taxa (see Appendix). We recorded 3467 visits during the study period. Interactive visits were made almost
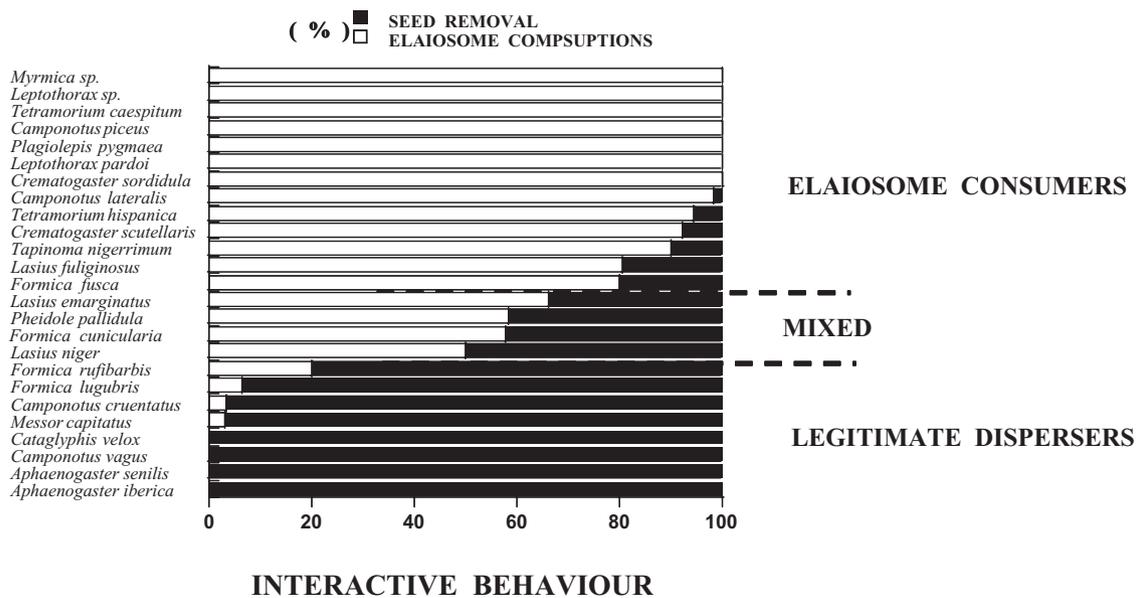
Figure 2. Interactive behaviour of the ant species using *Helleborus foetidus* diaspores (only ants with more than four interactive records included). The data depict the percentage of diaspore removals and elaiosome consumptions per ant species. The interactive behaviour was characterized over the total of interactive records of each ant species (range = 6–480).

entirely by ants (97.8%) and represented 50.6% of total visits, Despite the huge variation across populations, regions and years in the size and composition of the assemblage (see below), the ant–visitor assemblage was mainly represented by species belonging to subfamily Formicinae (genera *Camponotus*, *Formica*, and *Lasius*; see Appendix), that accounted for 72.7% of the ant interactive records. The rest of ant taxa of the assemblage were composed of subfamilies Myrmicinae, accounting for 25.72% (mainly genera *Aphaenogaster* and *Pheidole*; see Appendix), and Dolichoderinae, 1.58% (only genus *Tapinoma*) of the interactive records. Others arthropod taxa represented less than 2.25% of the interactive records, and were mainly beetles (Carabidae, Staphylinidae and Curculionidae), harvestmens (Opilion), mites (Tetranychidae), yellow-jacket wasps (genus *Polistes*) and bedbugs (Lygaeidae).

The interactive behaviour varied significantly among ant species (Wald's $\chi^2 = 93.7$, d.f. = 1,29, $P < 0.0001$) and remained constant for the same ant species across populations, regions or years for most ant species ($P > 0.05$ in all cases). Only *Formica cunicularia*, behaves differently between populations ($Z = 2.92$, d.f. = 1,6, $P = 0.0018$); whereas it was mainly an elaiosome consumer in Caurel (75% of nine interactive records), in Mágina, it showed a mixed behaviour (54% of seed removal, 46% of elaiosome consumption, $N = 11$).

Most ant species of the vistor assemblage of *H. foetidus* were elaiosome consumers, which accounted for 54.3% of 35 ant taxa (Fig. 2), whereas legitimate dispersers and mixed consumers accounted for 28.6% and 17.1%, respectively (Fig. 2). However, the ant–disperser assemblage was numerically dominated by legitimate dispersers and mixed consumers, as suggested by the number of plant visited per population, the mean relative frequency of visits, and the mean proportion of seeds removed (see Appendix). Finally, all other insect visitors were elaiosome consumers, but wasps, although anecdotal visitors (0.004% of the interactive visits), were able to remove the diaspore.

VARIATION IN SPACE

A total of 25 ant species were recorded visiting *H. foetidus* seeds or carpels at ten study sites (range = 4–13). The overall abundance and composition of ant assemblages varied markedly between populations and regions (see Appendix). The similarity in the assemblages between populations was generally low and not significant (PS = 0.19 ± 0.25, $P > 0.05$; PS ± 1 SD). Only six out of the 45 pairwise comparisons between sites indicated significant similar (i.e. PS significantly different from zero; Table 3) assemblage composition: four from comparison between populations within the same region and two from

Table 3. Proportional similarity (PS) between ant assemblages of *Helleborus foetidus*

Populations	Similarity									
	MTBJ	PM	BY	CAL	RH	RHW	LIN	TOR	CRU-1	CRU-2
MTBJ	–	0.0557	0.4452	**0.7865**	**0.3866**	0.125	0.748	0	0	0
PM		–	0.125	0.0617	0.0632	0.125	0.125	0	0.1154	0
BY			–	0.5889	0.3786	**0.2788**	0.5384	0	0	0
CAL				–	**0.4249**	0.125	0.7544	0	0	0
RH					–	0.1776	0.6	0	0	0
RHW						–	**0.3365**	0	0	0
LIN							–	0	0	0
TOR								–	0.0897	0
CRU-1									–	**0.7949**
CRU-2										–

A bootstrap resampling procedure was used to estimate significance levels of PS values. Significant PS values ($P = 0.05$) are shown in bold.

Mágina: MTBJ, Matabejid; PM, Puerto de la Mata. Cazorla: BY, Barranco de la Yedra; CAL, Calvario; RH, Roblehondo; RHW, Roblehondo-Forest; LIN, Linarejos. Peña Negra: TOR, Barranco del Toril; CRU-1, Caurel: Las Cruces-1; CRU-2, Las Cruces-2.

populations of nearby regions (Mágina and Cazorla; Table 3, Fig. 1), suggesting a distance-dependent similarity pattern. A significant Mantel's test corroborated this extreme ($r = 0.63$, $P = 0.0014$).

The relative frequency of the three groups of behaviour differed significantly in space. The frequency of legitimate dispersers varied significantly among populations ($Z = 5.5$, d.f. = 3,3, $P = 0.0009$) but not among regions ($F = 0.95$, d.f. = 3,6, $P = 0.47$). Although they were especially abundant in Las Cruces-2 and Roblehondo, reaching values above 70% of visitors (Fig. 3), they scarcely accounted for 15% of the visitors in Barranco del Toril, Roblehondo-Forest, or Puerto de la Mata (Fig. 3). Similarly, elaisome and mixed consumers differed among populations (elaisome consumers: $Z = 3.10$, d.f. = 3,3, $P = 0.007$; mixed behaviour: $Z = 3.23$, d.f. = 3,3, $P = 0.005$) but not among regions (elaisome consumers: $F = 1.20$, d.f. = 3,6, $P = 0.39$; mixed behaviour: $F = 0.81$, d.f. = 3,6, $P = 0.54$). The proportion of elaisome consumers scored minimum in those populations where the frequency of legitimate dispersers was high (e.g. Las Cruces-2 or Roblehondo; Fig. 3). A reverse circumstance occurred in Roblehondo-Forest, Barranco del Toril, or Puerto de la Mata, where elaisome consumers were nearly 50% (Fig. 3).

TEMPORAL VARIATION

We recorded 12 ant species in Barranco de la Yedra and 11 in Calvario in 2001–03 (range = 9–10 and 6–9, respectively). The assemblage composition did not differed significantly among consecutive years at each population (Table 4). Thus, both in Barranco de la Yedra and Calvario, the interannual similarity in assemblage species composition was high and significant (PS = 0.64 ± 0.08; PS = 0.80 ± 0.12).

However, the relative frequency of each behaviour varied significantly through the years at each population: legitimate dispersers (Barranco de la Yedra: Wald's $\chi^2 = 303.7$, d.f. = 2,48, $P < 0.0001$; Calvario: Wald's $\chi^2 = 113.9$, d.f. = 2,46, $P < 0.0001$; elaisome consumers (Barranco de la Yedra: Wald's $\chi^2 = 240.6$, d.f. = 2,48, $P < 0.0001$; Calvario: Wald's $\chi^2 = 36.6$, d.f. = 2,46, $P < 0.0001$); and mixed behaviour (Wald's $\chi^2 = 242.3$, d.f. = 2,48, $P < 0.0001$; Wald's $\chi^2 = 55.9$, d.f. = 2,46, $P < 0.0001$, respectively). At Barranco de la Yedra, elaisome consumers were the most abundant group in 2001 and 2003 (Table 5), whereas legitimate disperser dominated the ant–disperser assemblage in 2002. At Calvario, however, legitimate disperser was the most representative group through the study period.

PARTITION OF THE SPATIO-TEMPORAL VARIATION

Overall, the spatial variation of each interactive group of ant–visitor assemblage was higher than the temporal variation (Table 6). However, most variance in space was due to variation among populations rather than to variation among regions, which was
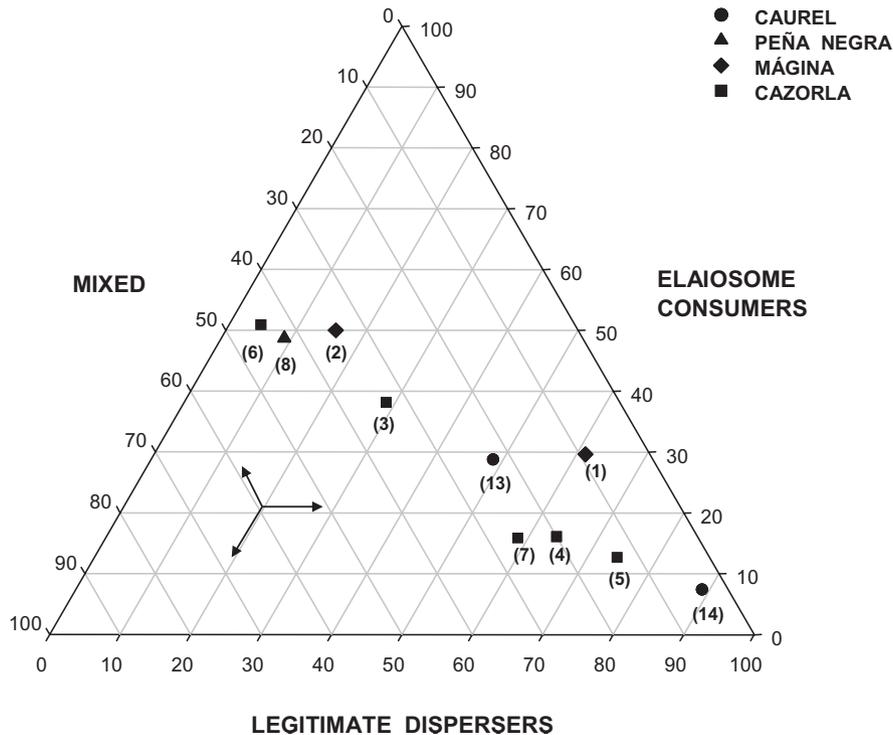
Figure 3. Ternary graph showing the variation across the Iberian Peninsula in the function (percentage of three interactive behaviours) of the ant assemblages in *Helleborus foetidus*. Numbers in parenthesis under each symbol identify the study populations (Table 1). Arrows within the graph denote the reading address for each axis.

Table 4. Inter-annual proportional similarity (PS) in ant assemblages of *Helleborus foetidus* in two populations from Cazorla

Population	Year	2001	2002	2003
Barranco de la Yedra	2001	–	0.655	0.698
	2002	–	–	0.551
	2003	–	–	–
Calvario	2001	–	0.708	0.771
	2002	–	–	0.946
	2003	–	–	–

A bootstrap resampling procedure was used to estimate significance levels of *PS* values. All *PS* were significant ($P = 0.05$).

negligible. This was especially pronounced for legitimate dispersers and elaiosome consumers (Table 6). Temporal variation also accounted for significant variance in relative abundance of the three interactive groups, particularly for the mixed behaviour (Table 6).

DISCUSSION

TAXONOMIC AND FUNCTIONAL DIVERSITY OF THE ASSEMBLAGE

Most visitors of *H. foetidus* diaspores were ants. The ant–visitor assemblage of *H. foetidus* was diverse, according to the great variety of habitats and the broad geographical range considered in the present study, which redound upon a great variety of ecological conditions. The assemblage was chiefly made up of species of the subfamily Formicinae. Some genera such as *Camponotus*, *Formica*, or *Lasius* were present in almost all populations and years. Specifically, most visits and dispersal events were conducted by *Camponotus cruentatus* in southern populations or *Formica lugubris* in the north of the Iberian Peninsula (see Appendix). Species of *Lasius* genus (*Lasius niger*, *Lasius emarginatus*, or *Lasius fuliginosus*) were also abundant, numerically dominating some assemblages (e.g. in localities from central Iberian Peninsula; see Appendix). The ant–disperser assemblage was completed by myrmicines such as *Aphaenogaster iberica*, *Aphaenogaster senilis*, *Tetramorium hispanicum*, *Tetramorium caespitum*, *Pheidole pallidula*, and several *Myrmica* and *Leptothorax* species

Table 5. Average relative frequency (%), estimated by a generalized linear model, of three types of interactive behaviour

Population	Interactive group	Relative frequency (%)			Post-hoc tests (χ^2)		
		2001	2002	2003	2001–2	2001–3	2002–3
Barranco de la Yedra	Legitimate	19.43	50	28.71	261.31	66.46	105.79
	Mixed	15.34	11.60	33.15	26.27	66.34	211.35
	Elaiosome consumers	65.23	38.40	38.14	156.48	212.36	5.38
Calvario	Legitimate	57.70	76.16	63.90	102.32	13.86	62.81
	Mixed	23.32	12.75	20	48.74	4.76	33.77
	Elaiosome consumers	18.98	11.09	16.10	34.35	5.95	18.10

Data come from two populations from Cazorla during three years. All χ^2-values of post-hoc tests were statistically significant ($P < 0.05$).

Table 6. Variance partitioning on the frequency of each interactive behaviour between regions, populations and years

	Variation								
	Spatial						Temporal		
	Region			Population			Year		
Interactive group	% Var	Z	P	% Var	Z	P	% Var	Z	P
Legitimate	0.15	0.58	0.280	**82.73**	1.47	0.031	**17.11**	1.95	0.025
Mixed	0.88	0.68	0.249	**51.01**	1.05	0.046	**48.11**	1.93	0.027
Elaiosome consumers	0.22	0.97	0.166	**73.81**	1.36	0.047	**25.95**	1.98	0.024

Percentages of variance (% Var) differing significantly from zero are shown in bold.

(see Appendix). The ant assemblage of *H. foetidus* did not differ substantially from other european myrmecochorous herbs, both from Mediterranean (Wolff & Debussche, 1999; Garrido *et al*., 2002; Gómez *et al*., 2003) and from temperate regions (Gorb & Gorb, 1999, 2003; Peters, Oberrath & Böhning-Gaese, 2003).

In parallel with the great species diversity in the assemblage, we observed functional diversity. More than half of the ant species of the assemblage were elaiosome consumers, which interfered with the dispersal mutualism, obtaining a reward without any compensation to the plant. Nonetheless, legitimate dispersers and mixed consumers dominated quantitatively the disperser assemblage. The variety in interactive behaviour of the ant assemblages appears to be the norm in other ant–seed dispersal systems (Pudlo *et al*., 1980; Horvitz & Schemske, 1986; Gorb & Gorb, 1999; Gómez *et al*., 2003). The interactive behaviour of each ant species was highly consistent among populations and regions (but see *F. cunicularia*), meaning that its functional role did not depend on the ecological context. This result contrasts with other findings that documented shifts within species in interactive behaviour in relation to shifts in the competitive scenario where the interaction takes place (Gorb & Gorb, 1999, 2003). More information on other myrmecochory systems is thus needed to elucidate the commonness of such shifts in function on an ant species. Ant size has been noted as the main factor influencing the interactive behaviour with myrmecochorous seeds and seed dispersal success (Garrido *et al*., 2002; Ness *et al*., 2004). The influence of ant size on the probability of seed removal in *H. foetidus* has also been demonstrated (Garrido *et al*., 2002). These authors determined that the probability for an ant species behaving as legitimate disperser of this plant was closely related to mean ant size (i.e. the larger the ant species the higher the likelihood of removal in diaspore offerings). Therefore, ant size differences between species will most likely explain the differences found in this study with respect to the interactive behaviour. Other aspects of the ant-foraging behaviour may also account for differences in interactive behaviour. For example, the complex relationships between prey

(i.e. diaspore) and ant size and the workers recruitment ability, and its influence on probability of seed dispersal (Traniello, 1989; Gorb & Gorb, 1999), are badly known.

SPATIAL VARIATIONS IN THE ANT–VISITOR ASSEMBLAGE

The visitor assemblage of *H. foetidus* varied notably among populations and regions. Such variations occurred both in diversity and species composition. The richest assemblages appeared at southern populations such as Matabejid (Mágina), Roblehondo, and Barranco de la Yedra (Cazorla), whereas the poorest ones occurred at Las Cruces-2 (Caurel) and at Barranco del Toril (Peña Negra). Composition of the assemblage also varied among spatial scales, following a distance-dependent pattern. Overall, assemblages from close populations and/or regions (e.g. Cazorla-Mágina) were more similar than far-distant populations and/or regions (e.g. Caurel-Cazorla or Caurel-Mágina; Table 3). Our results agree with previous studies that also reflected intrapopulational and interpopulational variations in the composition of the ant–disperser assemblages (Pudlo *et al*., 1980; Smith, Forman & Boyd, 1989; Hughes & Westoby, 1990; Wolff & Debussche, 1999). Thus, our results obtained over a wide geographical range, together with previous data, suggest that the spatial incongruities in the ant–disperser assemblages are the norm in the ant–seed dispersal mutualism.

Structuring factors that govern spatially ant communities and control ant diversity within locality will likely be the factors that control the spatial variation in the ant disperser guilds found in the present study. At a regional scale, temperature has been shown to be the main factor regulating the local diversity of ants (Kaspari, Ward & Yuan, 2004). Also, temperature appears to be responsible for the generation of latitudinal patterns of species richness and composition in ant assemblages (Cushman, Lawton & Manly, 1993). At a local scale (i.e. within region), a wide set of frequently interrelated processes, such as interspecific competition (Andersen, 1992; Cerdá *et al*., 1998; Sanders & Gordon, 2003), habitat complexity (Lassau & Hochuli, 2004), vegetation canopy (Feener & Schupp, 1998; Retana & Cerdá, 2000), temperature (Cerdá *et al*., 1998), and degree of anthropogenic disturbance (Andersen & Majer, 2004), have been proposed as community structuring forces. Presumably, some or all of these factors are also responsible for the spatial variation observed among *H. foetidus*'s ant assemblages.

Shifts in species composition of the assemblages among populations have produced concomitant variations in the relative frequency of the different types of interactive behaviour. Roblehondo-Forest, Barranco del Toril, and Peña Negra had the lowest proportion of legitimate dispersers and, subsequently, a high proportion of elaiosome and mixed consumers. The two firsts populations were located at dense woodlands, habitats where the well-developed canopy (Table 1) limits the structuring effect of temperature over Mediterranean ant communities (Retana & Cerdá, 2000). When the structuring effect of the temperature is relaxed, other factors, such as interspecific competition and dominance relationships, appear to be the main factors controlling ant communities (Retana & Cerdá, 2000). Often, dominant ants are small-sized and recruit massively to forage (Andersen, 1992), ants that, in the present study, typically behaved as elaiosome or mixed consumers. Accordingly, the disperser assemblage in these two populations was mainly carried out by small-sized species belonging to genera *Lasius*, *Crematogaster*, or *Pheidole*, with massive recruitment and behavioural dominance (Cerdá *et al*., 1998). The low frequency of legitimate dispersers at Puerto de la Mata was, however, due to both the ecological dominance of *T. caespitum* (small-sized ant species behaving as elaiosome consumer) and the absence of species of the genus *Camponotus* (see Appendix). In this population, only *Cataglyphis velox* and *Aphaenogaster senilis* acted as legitimate dispersers, but they jointly accounted for only 15% of interactive visits. The remainder of the populations had a high frequency of legitimate dispersers. These populations were located at sites with low vegetation cover, and temperature probably played a major role in structuring the ant communities (Cerdá *et al*., 1998), preventing small ants (normally the ecological dominants) accessing the resources. This effect of the temperature allows other subordinate ant species (more tolerant to high temperature) such as *C. cruentatus*, *Camponotus vagus*, *C. velox*, or *Aphaenogaster* spp., to access to *H. foetidus* diaspores. In northern areas (e.g. Caurel), interspecific competition and dominance among ant species are less dependant on temperature (Retana & Cerdá, 2000). The very high frequency of legitimate dispersers here (e.g. Las Cruces-2 with the maximum proportion of legitimate dispersers, approximately 90%) is due to the ecological success of *F. lugubris*, which monopolized the interactive events in this population. This ant belongs to *Formica rufa* group. Because of their aggressiveness and extreme territoriality, these ants occupy the top of the dominance hierarchy within the ant communities at northern habitats (Savolainen, Vepsäläinen & Wuorenrinne, 1989). They are large-sized ants (i.e. able to carry large seeds) with huge colonies that exclude other ants from their foraging territories (Savolainen *et al*., 1989).

In brief, the variation in size and species composition of the disperser assemblage of *H. foetidus* in the Iberian Peninsula translated to a great functional variation of the assemblage. At the expense of further analysis of the effects of such variation on dispersal success (i.e on seed removal, germination, and seedling recruitment), we can envisage a selection mosaic scenario (*sensu* Thompson, 2005) on dispersal-related diaspore traits, as suggested by Garrido *et al*. (2002), where local adaptive responses of plants might arise as a result of local variations in the specific composition and function of the assemblage.

TEMPORAL VARIATION IN THE ANT–VISITOR ASSEMBLAGE

The assemblage species composition did not differ among successive years within a site, suggesting the existence of a predictable interactive scenario over time, at least with respect to the identity of the dispersal agents. This congruent temporal pattern contrasts with the temporal variations found both in size and species composition in other myrmecochory systems (Horvitz & Schemske, 1990; Rico-Gray *et al*., 1998, 2004). Knowledge about the patterns of variation over time of the myrmecochores assemblages is still scarce, being mainly concerned with daily or seasonal variation rather than interannual variation (but see Gómez *et al*., 2003). However, interannual variations in the pattern of seed removal by ants have been shown in Australian rich myrmecochore areas (Hughes & Westoby, 1990), providing indirect evidence of between-years shifts in disperser assemblage. Such variations have been related to environmental parameters such as temperature, rainfall and/or moisture, factors that are also used to control the availability of plant resources for ants (Rico-Gray *et al*., 1998, 2004). These factors are also responsible for the structural shifts in ant communities over different scales of time (Retana & Cerdá, 2000; Albrecht & Gotelli, 2001). Diaspore release of *H. foetidus* takes place in June and July, when there are highly predictable extreme temperatures and scarce rainfalls in Mediterranean areas. Fruit maturation and seed shedding of myrmecochorous species has been shown to be adjusted to the period of maximum ant activity (Oberrath & Böhning-Gaese, 2002). This is also true in our system because seed release of *H. foetidus* overlaps with the period of maximum ant activity in our populations. Therefore, interannual consistency between successive years in the species composition of ant–disperser assemblage of *H. foetidus* is not an unexpected result. This interannual congruency of the disperser assemblage could succeed in a concomitant congruency in selection pressures on diaspore traits in each population, and presumably a consistent plant response to these pressures.

However, from a functional view, ant assemblages varied significantly among consecutive years within a population, although they did so in different ways depending on the population. In Calvario, differences among years only affected the magnitude of the relative frequency of each group of interactive behaviour, without modifying the sequence: legitimate dispersers > 'mixed' > elaiosome consumers (Table 5). In Barranco de la Yedra, these differences also affected the sequence of the three types of interactive behaviour. This variation suggests some stochasticity in the disperser assemblage function in the latter population. Because the interactive behaviour of each ant species is not likely to vary between years, the population fluctuation of the species of each interactive group (Herbers, 1989) must explain these temporal variations as the function of the assemblage. Although longer temporal series are needed, some unpredictability can be presumed regarding the interactive scenario, at least in some populations. This unpredictability arises from changes in the function of the ant assemblage (i.e. fluctuations in the relative frequency of the different groups of interactive behaviour) rather than from interannual shifts in the assemblage species composition. Noticeably, this could result in diffuse selection (Herrera, 1985; Horvitz & Schemske, 1990), especially if such fluctuations do not arise from deterministic patterns, as appears to be the case in Barranco de la Yedra.

MAGNITUDE OF THE SPATIO-TEMPORAL VARIATION IN THE ASSEMBLAGE

The magnitude of the spatial variation (mainly due to variation among populations) was higher than temporal variation for the three groups of interactive behaviour. This result suggests that the relative frequency of each functional group will be more predictable among years within each population than among populations. Unfortunately, no previous study has explored, simultaneously, the spatial and temporal variations in composition, shape, and structure of ant–disperser assemblages, although some indirect evidence is available. Hughes & Westoby (1990) described important spatial and temporal variations in the rates of removal of myrmecochore seeds (with spatial variation accounting for more variance). These authors attributed such variations to shifts in environmental parameters (mainly temperature) rather than shifts in the species composition or function of the disperser assemblage, Studies on ant–guard mutualisms have also shown the existence of notable variation, both in space and time, in the specific composition of the ant–guard assemblages (Horvitz

& Schemske, 1990; Rico-Gray *et al*., 1998; Rudgers & Gardener, 2004). Therefore, despite a lack of information, it appears that spatio-temporal incongruities could be the norm in seed dispersal by ants, although temporal variation would be more apparent in the function of the assemblage. If shifts in the ant–disperser assemblage translate to shifts in dispersal success, they could promote diverse scenarios of selection. Although selection by an assemblage taxonomically or functionally variable in space but constant in time could lead to local divergence and specialization (Herrera, 1988; Horvitz & Schemske, 1990), selection on plants by assemblages variable in time could lead to ecological generalization (Herrera, 1988; Gómez & Zamora, 1999).

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APPENDIX

ANT TAXA RECORDED ON REPRODUCTIVE *HELLEBORUS FOETIDUS* OVER 3 YEARS ACROSS
THE IBERIAN PENINSULA

	2001			2002			2003		
	FRV	SVP	PV	FRV	SVP	PV	FRV	SVP	PV
CAZORLA									
Barranco de la Yedra									
Ant species			(N = 12)			(N = 19)			(N = 20)
Aphaenogaster iberica	5.37	3.867	25	11.80	12.17	12.50	7.50	7.50	7.69
Camponotus cruentatus	14.04	16.17	*66.67*	*36.45*	*26.50*	*50.0*	*20.50*	20.31	*26.92*
Camponotus vagus	–	–	–	1.75	0.44	4.17	–	–	–
Camponotus lateralis	*20.63*	16.60	*66.67*	7.90	9.72	8.33	12.92	12.92	15.38
Crematogaster scutellaris	14.32	11.41	58.33	11.84	16.88	25.0	8.21	7.78	15.38
Leptothorax pardoi	20.43	21.92	50	5.30	5.502	8.33	7.0	7.63	11.54
Pheidole pallidula	15.34	*22.66*	50	11.58	11.305	20.83	19.82	*22.71*	23.07
Plagiolepis pygmaea	7.15	8.42	33.33	10.96	12.59	25.0	5.0	3.33	7.69
Tapinoma erraticum	1.19	0.25	8.33	2.40	4.197	8.33	–	–	–
Myrmica spp.	1.51	0.64	8.33	–	–	–	–	–	–
Lasius niger	–	–	–	–	–	–	13.33	13.33	11.54
Crematogaster sordidula	–	–	–	–	–	–	5.0	5.0	3.84
Formica subrufa	–	–	–	–	–	–	0.71	0.089	3.84
Calvário									
Ant species			(N = 11)			(N = 15)			(N = 23)
Aphaenogaster iberica	1.50	1.82	8.33	9.33	9.63	9.09	1.95	1.69	7.14
Camponotus cruentatus	*53.0*	*54.51*	*83.33*	*65.5*	*64.89*	*50.0*	*61.07*	*58.16*	*64.28*
Cataglyphis velox	3.20	2.49	16.67	–	–	–	0.87	1.00	3.57
Pheidole pallidula	23.32	28.89	*83.33*	12.75	10.02	18.18	18.35	18.34	30.55
Tetramorium hispanicum	17.56	11.72	50.0	6.611	6.04	13.64	6.48	7.57	23.24
Leptothorax sp.	1.80	0.56	8.33	–	–	–	–	–	–
Messor capitatus	–	–	–	1.33	2.22	4.54	–	–	–
Camponotus piceus	–	–	–	0.83	1.71	4.54	1.74	3.17	15.24
Crematogaster sordidula	–	–	–	3.63	5.48	4.54	1.26	7.61	25.49
Lasius niger	–	–	–	–	–	–	1.74	1.00	4.81
Crematogaster scutellaris	–	–	–	–	–	–	6.52	7.61	25.49
Roblehondo									
Ant species						(N = 22)			(N = 23)
Aphaenogaster iberica				2.77	2.77	7.69	–	–	–
Camponotus cruentatus				*41.95*	*41.64*	*50.0*	28.095	40.32	*27.44*
Lasius niger				10.30	11.35	15.38	10.72	30.18	12.69
Camponotus piceus				0.91	0.41	3.84	–	–	–
Crematogaster scutellaris				0.91	0.41	3.84	3.17	14.54	4.16
Camponotus vagus				16.67	16.47	19.23	19.05	37.0	18.41
Formica subrufa				0.75	0.56	3.84	9.52	30.07	9.52
Leptothorax pardoi				6.36	5.61	11.53	2.38	10.91	3.03
Cataglyphis velox				2.27	3.21	7.69	5.71	22.04	5.71
Tapinoma erraticum				0.91	1.94	3.84	–	–	–
Pheidole pallidula				2.02	2.02	7.69	2.38	10.91	3.81
Messor capitatus				14.16	13.56	26.92	11.82	22.8	8.05
Plagiolepis pygmaea				–	–	–	4.76	21.82	4.76
Tetramorium hispanicum				–	–	–	2.38	10.91	2.38
Roblehondo-Forest									
Ant species						(N = 14)			(N = 22)
Camponotus cruentatus				4.76	17.82	5.35	4.54	21.32	4.54
Camponotus lateralis				21.42	37.79	21.42	13.63	31.55	15.29

APPENDIX *Continued*

	2001			2002			2003		
	FRV	SVP	PV	FRV	SVP	PV	FRV	SVP	PV
Camponotus piceus				9.52	27.51	8.92	–	–	–
Crematogaster scutellaris				2.38	8.91	3.29	17.19	31.76	17.35
Camponotus vagus				9.52	27.51	10.43	–	–	–
Lasius niger				16.07	36.17	18.36	*43.41*	*39.59*	*45.24*
Leptothorax pardoi				*27.38*	*42.17*	*25.54*	18.56	30.85	15.67
Myrmica spp.				3.57	13.36	3.57	–	–	–
Pheidole pallidula				1.78	6.68	1.02	1.13	5.33	1.13
Plagiolepis pygmaea				3.57	13.36	2.04	–	–	–
Tetramorium hispanicum				–	–	–	1.51	7.10	1.51
Linarejos									
Ant species									(*N* = 17)
Camponotus cruentatus							*55.54*	*44.47*	*57.17*
Camponotus vagus							2.94	12.13	4.57
Crematogaster scutellaris							2.94	12.13	1.47
Tetramorium hispanicum							5.88	24.25	5.88
Leptothorax pardoi							5.88	24.25	5.88
Lasius niger							25.63	42.34	24.45
Plagiolepis pygmaea							1.17	4.85	0.56
MÁGINA									
Matabejid									
Ant species									(*N* = 30)
Aphaenogaster senilis							1.47	1.87	16.67
Camponotus cruentatus							*57.99*	*53.45*	*90.0*
Camponotus foreli							0.74	0.32	10.0
Tapinoma nigerrimun							6.71	7.41	10.0
Camponotus lateralis							1.08	0.61	6.67
Cataglyphis rosenhaueri							0.14	0.08	3.33
Crematogaster scutellaris							1.08	2.45	6.67
Crematogaster sordidula							12.11	16.09	33.33
Cataglyphis velox							0.33	0.50	6.67
Leptothorax pardoi							1.52	0.95	16.67
Messor capitatus							1.33	1.67	3.33
Pheidolle pallidula							9.13	8.82	3.33
Plagiolepis pygmaea							6.37	4.77	36.67
Puerto de la Mata									
Ant species									(*N* = 16)
Aphaenogaster senilis							3.12	3.12	3.45
Cataglyphis velox							12.5	12.5	6.89
Formica cunicularia							18.75	14.67	13.79
Tapinoma nigerrimun							5.21	3.82	6.89
Camponotus lateralis							15.62	15.62	10.34
Lasius niger							15.62	15.62	10.34
Tetramorium caespitum							29.16	34.72	20.69
PEÑA NEGRA									
Barranco del Toril									
Ant species						(*N* = 16)			(*N* = 13)
Lasius emarginatus				*53.81*	*53.65*	*34.48*	*42.31*	*44.61*	*23.33*
Formica fusca				31.19	29.22	24.14	26.92	26.92	13.33
Cataglyphis spp.				6.25	6.25	3.45	–	–	–
Leptothorax spp.				6.25	6.25	3.45	–	–	–

APPENDIX *Continued*

	2001			2002			2003		
	FRV	SVP	PV	FRV	SVP	PV	FRV	SVP	PV
Tetramorium hispanicum				2.50	4.33	3.45	14.10	12.82	10.0
Formica rufibarbis				–	–	–	8.97	7.95	6.67
Prado de la Francesa									
Ant species						(N = 14)			
Lasius emarginatus				*61.02*	*58.56*	*46.67*			
Formica fusca				7.14	7.14	3.33			
Formica rufibarbis				5.65	8.10	10.0			
Myrmica spp.				7.14	7.14	3.33			
Formica sanguinea				7.14	7.14	3.33			
Lasius fuliginosus				7.14	7.14	3.33			
Leptothorax spp.				4.76	4.76	3.33			
CAUREL									
Mostad									
Ant species			(N = 8)						
Formica lugubris	*75.0*	*75.0*	*60.0*						
Leptothorax spp.	25.0	25.0	30.0						
Entretaros									
Ant species			(N = 9)						
Formica lugubris	*50.0*	*51.97*	*60.0*						
Formica rufibarbis	22.22	20.25	30.0						
Lasius niger	27.77	27.77	30.0						
Las Cruces Abierto-1									
Ant species						(N = 17)			(N = 20)
Formica lugubris				17.64	17.64	10.0	*47.01*	*48.30*	*24.44*
Lasius emarginatus				14.71	14.71	10.0	6.21	7.21	6.67
Formica sanguinea				14.71	15.68	10.0	–	–	–
Lasius niger				1.96	1.96	3.33	4.05	3.11	6.67
Formica fusca				*31.37*	*30.39*	*23.33*	5.0	4.85	0.02
Leptothorax spp.				5.88	5.88	3.33	–	–	–
Tetramorium hispanicum				11.76	11.76	6.67	1.02	2.0	0.02
Tapinoma erraticum				1.96	1.96	3.33	5.0	5.0	0.02
Formica rufibarbis				–	–	–	3.7	4.2	*24.44*
Formica cunicularia				–	–	–	13.01	14.89	13.33
Myrmica spp.				–	–	–	15.0	10.45	0.11
Las Cruces Abierto-2									
Ant species									(N = 18)
Formica lugubris							*88.89*	*88.89*	*53.33*
Lasius emarginatus							3.70	5.25	3.33
Tetramorium hispanicum							5.55	5.55	3.33
Myrmica spp.							1.85	0.31	3.33
Las Cruces Bosque									
Ant species						(N = 13)			
Lasius fuliginosus				*69.23*	*69.23*	*30.0*			
Lasius emarginatus				23.07	23.07	10.0			
Myrmica spp.				7.69	7.69	3.33			

Only interactive visits are included. Maximum record of each variable in each population and year is shown in italics. The number of plants censused each year is indicated in parenthesis. Additional information will be provided by the authors upon request. FRV, relative frequency of visits (%); SVP, seeds visited per plant (%); PV, plant visited (%).