

Geographical variation in the determinants of seed dispersal success of a myrmecochorous herb

PEDRO J. REY*† and ANTONIO J. MANZANEDA‡§

†*Department Biología Animal, Biología Vegetal y Ecología, Área de Ecología, Universidad de Jaén, E-23071 Jaén, Spain*, ‡*Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avda. Maria Luisa s/n, E-41013 Sevilla, Spain*

Summary

1. This paper draws together geographical variation and various correlates of individual seed dispersal success to evaluate spatial variation in major determinants of seed removal rates of the myrmecochore *Helleborus foetidus*, whose ant assemblages and diaspores vary geographically.

2. We censused seed removal by ants directly from individual plants in 12 populations and four regions across the Iberian Peninsula, and measured the elaiosome seed, plant fecundity, and the biotic and abiotic environment in the close vicinity of each plant. The relative effect of these three groups of variables on seed removal was then modelled at each region using structural equation models and path analyses.

3. We found a pervasive effect of the close surroundings of each plant on dispersal. We also found a geographically variable influence of diaspore traits on dispersal. Such variable influence of the diaspore was closely related to the compositional and functional variation in ant guilds.

4. Synthesis: The appearance of an effect of diaspore and plant traits on dispersal depended on the surroundings of the plant, the identity of the ant visitors and ant abundance. This dependence itself might lead to different directions of selection among populations, a conclusion that has not emerged from seed offerings to ants, which is the usual protocol in myrmecochory research.

Key-words: ant–plant mutualisms, elaiosome-bearing seeds, hellebore, myrmecochory, path analysis, seed dispersal determinants, seed dispersal macroecology, structural equation models.

Journal of Ecology (2007) **95**, 1381–1393
doi: 10.1111/j.1365-2745.2007.01293.x

Introduction

Many plant species (more than 3000 in over 80 families) have their seeds dispersed by myrmecochory (Beattie & Hughes 2002), i.e. attracting ants with elaiosomes – lipid-rich bodies attached to the seeds. Studies on myrmecochory have frequently shown that both individual ant species and assemblages are able to choose among seeds of different species based on seed and/or elaiosome size (Oostermeijer 1989; Hughes & Westoby 1992; Mark & Olesen 1996; Christian 2001; Peters *et al.* 2003; Edwards *et al.* 2006). Similarly, many

studies have independently considered other biotic and abiotic correlates of dispersal success, including temperature (Ness & Bressmer 2005), availability of alternative food (Boulay *et al.* 2005), elaiosome chemistry (Boulay *et al.* 2006, 2007), and timing of seed fall and the interference of other consumers with dispersal (Ohkawara *et al.* 1996, 1997; Fedriani *et al.* 2004). Detailed information on the ecology of myrmecochory is thus available, but the consequences of the interaction for the individual plant dispersal performance are poorly known (but see Kalisz *et al.* 1999). This is because, unlike studies on the ecological and evolutionary consequences of other plant–animal mutualisms, such as pollination or seed dispersal by vertebrates, studies on myrmecochory have not been based on inter-individual variation in, and covariation between, traits and dispersal (seed removal) success

(but see Mark & Olesen 1996; Boulay *et al.* 2007). As such, conclusions on the ecological and evolutionary consequences of myrmecochory are necessarily limited (Giladi 2006).

Moreover, ant assemblages may differ along the distribution range and through the different environments of one and the same myrmecochorous plant (Manzaneda *et al.* 2007). However, few macroecological reports show large-scale variation in a given ant–plant interaction (but see Garrido *et al.* 2002). In fact, how variation in ant assemblages correlates with seed dispersal within species has been considered only at the local scale (Hughes & Westoby 1990).

An emerging view is that the consequences of an interaction with animals on the ecology and evolution of the plant should be examined in conjunction with other biotic (competition and other interactions with animals) and abiotic components of the environment (Herrera 1993, 1995, 2000; Caruso 2000; Herrera *et al.* 2002; Strauss & Irwin 2004; Rey *et al.* 2006). Few studies on seed dispersal by animals (Jordano 1995; Alcántara *et al.* 2000), and none on myrmecochory, have adopted this view. This seriously hinders our knowledge of the influence of seed and other plant traits, relative to environmental factors, on dispersal performance.

This study brings together geographical variation in disperser assemblages and the various correlates of seed dispersal success in order to evaluate the spatial variation in the major determinants of the early dispersal performance in *Helleborus foetidus* (hereafter hellebore). Based on field observations of seed removal rates (after disperser visit censuses to reproductive plants), this paper evaluates the joint contribution of seed traits, plant fecundity and the environment immediately surrounding the plant, to inter-individual variation in seed removal by ants under different ant assemblage scenarios. To this end, path analysis and structural equation modelling were used to address two major questions: (i) What is the contribution of diaspore traits, compared with plant fecundity traits and environmental features, to the inter-plant variation in seed removal rates? (2) After accounting for the other type of determinants, is the effect of the diaspore on the inter-plant variation in early dispersal success geographically and temporally consistent, or does the influence of the seed traits on dispersal vary in relation to functional differences of the ant assemblages?

Methods

STUDY SPECIES AND STUDY AREA

Helleborus foetidus (Ranunculaceae) is a perennial herb distributed throughout central and southern Western Europe (Werner & Ebel 1994). It is common in clearings, patchy scrublands, forest edges, and the understorey of deciduous and mixed woodlands. In the Iberian Peninsula, it grows mainly at middle to high elevations in mountain areas of the north and south-

east. Plants have one or several reproductive ramets, each of which produces a single terminal inflorescence after several seasons of vegetative growth. Flowers have 1–6 carpels, with 10–15 elaiosome-bearing seeds. Diaspore (seed plus elaiosome) fresh mass ranges from 5 mg to 23 mg. The whole seed range can germinate, but germination rate is lower for the smallest seeds (our unpublished data). The elaiosome is soft and white, and comprises ~3–15% of the total diaspore fresh mass. It remains attractive to ants for 1–3 days after seed shedding. Fruit maturation and seed shedding take place in June–July. Ants are attracted by the elaiosome and carry the diaspores to the nest, where the elaiosome is removed and eaten. The intact seed may then be discarded within the nest or on a waste-midden on the surface. Some ants consume the elaiosome without carrying the seed, acting as elaiosome predators rather than as dispersers (note that the term ant is used here generically). The ability to act as seed dispersers or elaiosome predators is related to the ratio of ant size to diaspore size in this species (Garrido *et al.* 2002), but even small ants carry small diaspores and sometimes recruit workers to carry the largest ones. Rodents are also attracted by the seeds, always acting as seed predators. Ant removal occurs faster than rodent predation, but the opposite is also possible and causes interference with seed dispersal mutualism (Fedriani *et al.* 2004).

The study was conducted in May–June 2002 and 2003 in 12 populations (Fig. 1) in four distant regions of the Iberian Peninsula: Sierra de Cazorla and Sierra de Mágina (southern Spain), Peña Negra (near Sierra de Gredos, central Spain) and Sierra del Caurel (north-western Spain). All the populations studied within a region (five in Cazorla, two in Peña Negra and three in Caurel) were within 1 km of each other, except the two of Mágina, which were within 2.5 km. However, the distance between regions was large enough (within a range of 750 km) to explore geographical variation. In 2002 only Caurel, Peña Negra and Cazorla were sampled, while in 2003 Caurel, Cazorla and Mágina were sampled. The density of reproductive adults ranged between 0.6 per 100 m² in some populations of Cazorla to 6.5 per 100 m² in some populations from Mágina and Caurel.

INDIVIDUAL DISPERSAL PERFORMANCE

We chose between 22 and 45 reproductive hellebores at each population in each study year (Table 1). We estimated the seed removal rate by ants for each plant by observing ant activity and counting the number of seeds removed during 5-min censuses. We conducted between 6 and 22 censuses for each plant (3837 censuses on 492 plants in total). Censuses in each population were distributed over 2 or 3 weeks throughout the seed release period of the species (June–July). Censuses on the individual plants were distributed evenly in the population, and between 09:00 and 22:00 h GMT,

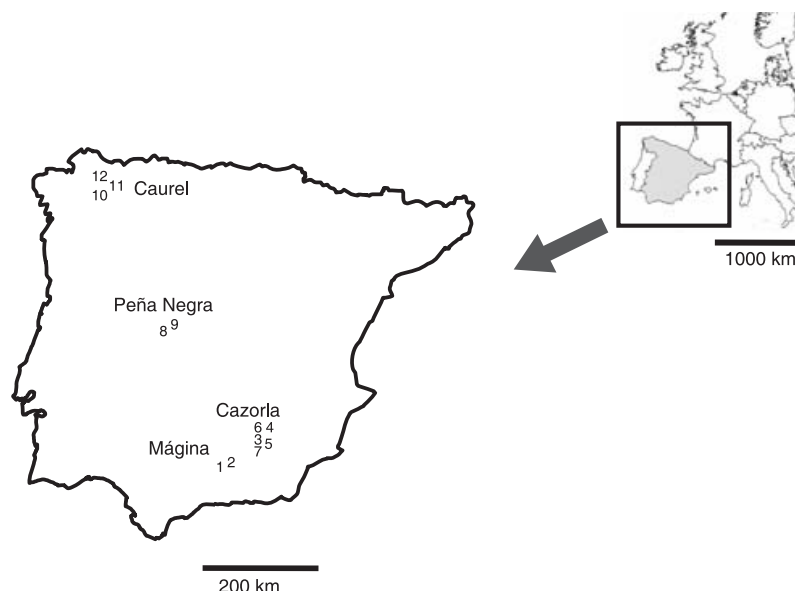


Fig. 1. Map of the Iberian Peninsula (left) showing the location of the 12 study populations. The two most distant regions (Caurel and Mágina) were ~760 km apart, while the two closest regions (Mágina and Cazorla) were ~85 km apart (see text for details). Number code and geographical coordinates for each population are: Matabejid (1: 37°43' N 3°30' W); Puerto de la Mata (2: 37°44' N 3°28' W); Barranco de la Yedra (3: 37°58' N, 3°54' W); Calvario (4: 37°58' N, 3°53' W); Roblehondo (5: 37°56' N, 3°52' W); Roblehondo-Forest (6: 37°56' N, 3°52' W); Linarejos (7: 37°56' N, 3°55' W); Barranco del Toril (8: 40°26' N, 5°18' W); Prado de la Francesa (9: 40°26' N, 5°18' W); Las Cruces-Forest (10: 42°36' N, 7°6' W); Las Cruces 1 (11: 42°36' N, 7°6' W); Las Cruces 2 (12: 42°36' N, 7°6' W). See also Table 1 for details.

according to a random permutation scheme. Censuses were always conducted in similar good weather conditions. In each census, the removal rate was calculated as the number of removed seeds divided by the available number of seeds per plant in the census. Since most ants were able to climb up the plant and retrieve the seeds directly from dehisced carpels, the total number of available seeds during each census was obtained by adding the number of seeds on the ground and the number of seeds on dehisced carpels on the plant. The total number of dispersed seeds for each maternal plant was estimated as the product of its seed crop (see below) and the seed removal rate, averaged through censuses. This value constitutes our estimate of early dispersal performance. Every census recorded the ant species visiting the plant and their interactive behaviour, distinguishing between dispersal and elaiosome consumption. Based on the frequency of these two behaviours, ants were functionally classified as: (i) legitimate seed dispersers – ants which behave predominantly as seed removers (i.e. when records of seed removal were $\geq 75\%$ of their total interactive records); (ii) elaiosome consumers – ants which behave predominantly as elaiosome predators (i.e. when records of *in situ* elaiosome consumption were $\geq 75\%$ of their total interactive records); and (iii) facultative dispersers – ants which behave indistinctly (i.e. records of seed removal and elaiosome consumption were in similar proportions).

Hellebore ant assemblages (species composition, abundance and importance of each functional behaviour) in these populations have been described

elsewhere (Manzaneda *et al.* 2007). Dominant species and relative representation of the legitimate dispersers in the ant assemblages are shown in Table 1. Combining information on species-specific ant size (data from Garrido *et al.* 2002) and the relative frequency of visits to hellebore diaspores of each ant species at each population (Manzaneda *et al.* 2007), we estimated an assemblage-level ant size (hereafter ASL-ant size) as the mean size of the species visiting hellebore weighted by the frequency of visits of each ant species.

FECUNDITY, DIASPORE AND ENVIRONMENTAL FEATURES

Each individual plant was characterized in relation to three major sets of traits. The first group was related to the ecological environment surrounding the plant during the period of seed release and included: distance to the closest reproductive neighbour (Dneighbour); mean temperature (recorded close to the carpels during the ant censuses using a digital thermometer; VAISALA® HM34); ant abundance (AntAb; estimated during ant censuses from the mean number of total ant workers on the plant and below the inflorescence projection); and interference of elaiosome consumers (Interference; estimated from the mean proportion of available seeds that lacked the elaiosome over the censuses). The second group was composed of fecundity traits, including: plant size (Plsize), which was estimated as the number of vegetative ramets; distance of the fruits from the ground (Dground); seed release synchrony (Synchrony), estimated from the coefficient of variation

Table 1. Short description of study sites. The number of plants used at each population and some characteristics of the ant-disperser assemblage from each population and year are given. Mean diaspore sizes (\pm SE) are also shown.

Region	Population (name abbreviation)	Habitat type	No. of plants*	Main ant visitors†	Legitimate dispersers visits* (%)	Diaspore size (mg)*
Mágina	Matabejid (MBJ)	Mediterranean open scrubland	-/30	<i>Camponotus cruentatus</i> (L) <i>Crematogaster sordidula</i> (C)	61.3	-/13 \pm 0.2
	Puerto de la Mata (PM)	Mediterranean open scrubland	-/29	<i>Tetramorium caespitum</i> (C) <i>Formica cunicularia</i> (M)	15.6	-/13 \pm 0.3
Cazorla	Barranco la Yedra (BY)	<i>Pinus</i> and <i>Quercus</i> mixed forest	24/26	<i>Camponotus cruentatus</i> (L)/ <i>C. cruentatus</i> (L) <i>Crematogaster scutellaris</i> (C)/ <i>Pheidole pallidula</i>	50.0/28.7	12 \pm 0.3/12 \pm 0.3
	Calvario (CAL)	Mediterranean open scrubland	22/28	<i>Camponotus cruentatus</i> (L)/ <i>C. cruentatus</i> (L) <i>Pheidole pallidula</i> (F)/ <i>Pheidole pallidula</i> (F)	76.2/63.9	12 \pm 0.3/13 \pm 0.2
	Roblehondo (RH)	<i>Pinus</i> and <i>Quercus</i> mixed forest	26/34	<i>Camponotus cruentatus</i> (L)/ <i>C. cruentatus</i> (L) <i>Camponotus vagus</i> (L)/ <i>C. vagus</i> (L)	78.7/74.2	11 \pm 0.5/13 \pm 0.3
	Roblehondo-Forest (RW)	<i>Pinus</i> and <i>Quercus</i> mixed forest	24/29	<i>Leptothorax pardoi</i> (C)/ <i>Lasius niger</i> (F) <i>Lasius niger</i> (F)/ <i>Leptothorax pardoi</i> (C)	14.3/4.5	14 \pm 0.4/15 \pm 0.2
Peña Negra	Linarejos (LIN)	<i>Pinus</i> and <i>Quercus</i> mixed forest	-/26	<i>Camponotus cruentatus</i> (L) <i>Lasius niger</i> (F)	58.5	-/15 \pm 0.4
	Barranco del Toril (TR)	<i>Quercus pyrenaica</i> forest	29/30	<i>Lasius emarginatus</i> (F) <i>Formica fusca</i> (C)	0.0/9.0	17 \pm 0.4
	Prado la Francesa (PF)	<i>Quercus pyrenaica</i> forest	30/-	<i>Lasius emarginatus</i> (F) <i>Formica rufibarbis</i> (L)	5.7	18 \pm 0.3/-
Caurel	Las Cruces-Forest (CrW)	<i>Pinus sylvestris</i> plantation	30/-	<i>Lasius fuliginosus</i> (F) <i>Lasius emarginatus</i> (F)	0.0	15 \pm 0.3/-
	Las Cruces 1 (Cr1)	Open scrubland pasture	30/45	<i>Formica fusca</i> (C)/ <i>Formica lugubris</i> (L)/ <i>Formica rufibarbis</i> (L)	17.7/48.5	14 \pm 0.3/16 \pm 0.3
	Las Cruces 2 (Cr2)	Open scrubland pasture	-/30	<i>Formica lugubris</i> (L) <i>Tetramorium hispanicum</i> (C)	88.9	-/17 \pm 0.7

*First cell-number (before the slash) is for 2002; second cell number is for 2003.

†L, F and C indicate Legitimate disperser, Facultative disperser and Elaiosome consumer, respectively.

of the instantaneous seed availability; mean seed number per carpel (Scarpel), estimated from 5–10 carpels per individual; and seed crop (Crop), estimated as the product of total carpel number per individual and the mean seed number per carpel. The third group was composed of diaspore-related traits, including: diaspore size (DS), which was estimated as the mean diaspore mass in mg from a bulk of all the seeds in 5–10 carpels per individual (both fresh and dry mass weighed with a METTLER TOLEDO® XS105 precision scale); and the elaiosome/seed ratio (E/S, representing the proportion of reward for the ant in relation to the ballast in the diaspore). These two variables are the best and the least redundant descriptors of hellebore diaspore (Garrido *et al.* 2002) after tolerance analysis in multiple regression.

DATA ANALYSES

A decomposition of variance of diaspore traits between regions, between populations within regions and

between plants was conducted separately for each year using a nested design. Population and region were treated as random effects. Analyses were conducted with the option COVTEST of the MIXED procedure of SAS. The model was fitted with restricted maximum likelihood, which is the most appropriate fitting procedure for the unbalanced nature of this type of design (Quinn & Keough 2002).

Diaspore-related dispersal correlates were examined by multiple linear regressions for each population and year. The dependent variable in these analyses was seed removal rate from a plant relative to the mean removal rate of the population. Diaspore traits were standardized to mean 0 and variance 1. We obtained regression coefficients and their 95% confidence intervals by bootstrapping, and we considered that a coefficient was statistically significant if its interval did not include zero. We tested for differences on dispersal correlates (between populations of a region and between years within region) with ANCOVA.

Because across-population variation in diaspore traits, ant size and dispersal can be inherently spatially structured, we examined distance dependence of these variables with Mantel's permutation test (Manly 1997; 10 000 iterations). This test correlated a between-populations pairwise dissimilarity matrix of a target variable with a geographical distance matrix. We further examined the relationship across populations between diaspore traits and dispersal after controlling for geographical distance using a partial Mantel test (Manly 1997). Mantel tests were conducted using a ZT program (Bonnet & Van der Peer 2002).

To assess the contribution of diaspore traits to dispersal performance relative to the contribution of plant-fecundity traits and surroundings, we used structural equation modelling (SEM) and path analysis. SEM is an increasingly widespread analytical tool in evolutionary ecology that allows the various causal relationships between a group of predictor variables (e.g. traits) and a target variable (the performance; see reviews in Scheiner *et al.* 2000; Pugesek *et al.* 2003) to be explored simultaneously. We built a causal scheme with four groups of variables for each region and year. The dispersal performance was the model target variable; we consider dispersal performance as a latent endogenous variable generated from an observed (manifest) variable (our estimate of the number of dispersed seeds for each plant). Because our interest is to assess the relative contribution to performance of three types of characters (diaspore traits, fecundity traits and surroundings features), a latent construct (not measured directly) was generated for each character type (Diaspore, Plant and Surrounding in the model, Fig. 2). Traits constructing each of these latent variables were those that correlate significantly with seed removal at some region or year of study, and appear in the model as manifest (i.e. measured variable) endogenous. These three latent variables were allowed to point through a direct pathway to the performance

latent variable. In all cases, the three latent constructs were included in the model as exogenous variables (i.e. variables not receiving any effect from another variable), following the model building proposed in Pugesek *et al.* (2003). The latent 'Plant' was constructed from a group of variables including plant crop, mean number of diaspores per carpel, plant size, carpel shedding synchrony and carpel distance to the ground. The latent 'Diaspore' was constructed from diaspore size and elaiosome to seed ratio. The latent 'Surrounding' was constructed from nearest-neighbour distance, ant abundance and interference. Finally, due to potential trade-offs between fecundity and diaspore traits, we allowed a correlation path between 'Plant' and 'Diaspore'. However, we did not trace a correlation path between latent 'Surrounding' and 'Plant' or 'Diaspore', because competitive or vicinity effects on fecundity were unlikely due to the low density of reproductive adults in each population (see Study area). Demography data (J.M. Ramírez & P.J. Rey, unpublished data) also suggest that plant fecundity is not affected by the density of adults in most populations. The proposed causal model for early dispersal performance is shown in Fig. 2.

The hypothesis of causal relationships of the model was tested through a chi-square goodness of fit test, which compares the observed covariance matrix with that expected from the relationships shown in the model (Shipley 1997). Small sample sizes make it less likely for the hypothesis of a good model fit to be rejected, so we used Steinger's RMSEA index (see Tomer & Pugesek 2003). This is suitable for providing evidence that the model is not a poor approximation, but a good one (values under 0.1 indicate that the model does not fit poorly, and values under 0.05 indicate close fit). The variables were log-transformed for normality and linearity, although some did not achieve normality after transformation. Thus, we used generalized least squares (GLS) as a discrepancy

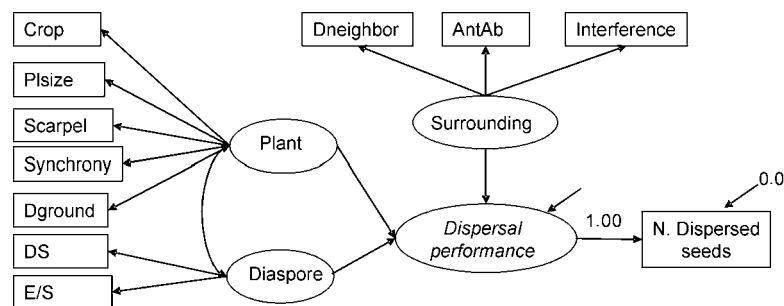


Fig. 2. Causal scheme for the variation in dispersal performance. Three groups of variables (plant fecundity, diaspore traits and surrounding features) are proposed to affect dispersal performance directly (see abbreviations of variable names in Methods). The effect of these groups of measured variables is summarized in a latent construct. Presumed life-history trade-offs between plant fecundity and diaspore traits are modelled through a correlation path (represented by a double-headed arrow) between Plant and Diaspore latent constructs. The latent variables (not directly measured) are shown within an ellipse, whereas manifest variables (those directly measured) are shown within a rectangle. Dispersal performance is a latent construct, and was estimated only from its surrogate seed removal. Consequently, the path from dispersal performance to number of dispersed seeds is forced to be 1.00 and the error of the latter is kept as 0. Error of Dispersal performance is indicated by a free arrow pointing to the latent construct. Model building follows that proposed by Pugesek *et al.* (2003).

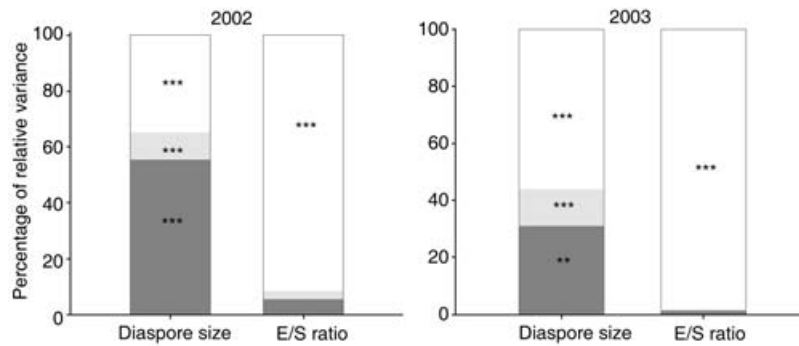


Fig. 3. Variance partitioning between regions (dark grey), populations within each region (light grey) and plants (white) in hellebore diaspore traits for each year. *** $P < 0.001$, ** $P < 0.05$.

function, which is preferred to maximum likelihood (ML) when the requirement of multinormality is not met (SAS Institute 1990). The analyses were conducted with the SEPATH module of STATISTICA v.6.0 (StatSoft 2000).

When building a model for a region we used the group option (see Grace 2003), which allowed us to incorporate data per population, treating all plants from each population as a group. This was because some evidence for differences in dispersal correlates between populations (see Results). We tested for a common general structure of the model for all populations of the same region by initially allowing all coefficients to differ among groups. This is the habitual starting point to compare groups (Grace 2003; Rey *et al.* 2006). We found a common structure among populations for all regions and years (goodness of fit test, $P > 0.50$ in all cases, indicating that the populations of the same region collectively fit a common model). We then tested for equality of each path coefficient between populations of the same region by starting with the assumption of total equality of path coefficients (all paths constrained to be equal between groups) and sequentially relaxing equality constraints (for an extensive review of procedures of multigroup comparison of path coefficients, see Grace 2003). If we place an equality constraint on a parameter across groups, the result is similar to the average of the respective estimates we would obtain without this parameter constraint. We can formally compare models with and without constraints by calculating the difference between the two chi-squares, and testing this difference against the chi-square distribution with one degree of freedom. Overall, we found few coefficients differing significantly between populations of the same region (see Results). Yet, the comparison between populations, which is limited by an insufficient number of cases per population, is not a major objective of this study. Instead, we were concerned with building a general model for each region. This is because the small distance between populations within region (often less than 1 km) probably allowed for considerable gene flow. The target variable (dispersal performance) was standardized to the population mean in order to obtain comparable estimates.

Results

VARIATION IN DIASPORE TRAITS

Diaspore size (DS) significantly varied at the three spatial scales for both years; however, the E/S ratio varied only significantly between plants within a population in both years (Fig. 3). Population always contributed to the total variance in DS much less than region and plant. In 2002, the region with the largest diaspores was Peña Negra (18 ± 0.3 mg), while the smallest diaspores came from Cazorla (12 ± 0.2 mg). At the population level, diaspores from Prado de la Francesa (Peña Negra) were the largest, while the smallest diaspores came from Roblehondo (Table 1; see within-population distribution of diaspore size in Fig. 4). In 2003, the region with largest diaspores was Caurel (16 ± 0.3 mg), while Mágina (12 ± 0.2 mg) had the smallest diaspores. That year, the largest diaspores came from Las Cruces 2 (Caurel), and the smallest from Barranco de la Yedra (Cazorla). In short, there was geographical variation in diaspore size: the central (Peña Negra) and northern (Caurel) localities had larger diaspores than the southern ones (Cazorla and Mágina). This was partly confirmed by the fact that, at least during the second year of study, the closer two populations were, the more similar were their diaspore traits (Mantel test, $r = 0.64$, $P = 0.01$ for DS; $r = 0.53$, $P = 0.03$ for E/S ratio). These relationships were also positive, but not significant, in 2002 ($r = 0.11$, $P = 0.25$ for DS; $r = 0.18$, $P = 0.18$ for E/S).

ANT ASSEMBLAGES

Twenty-five ant species visited hellebore seeds at our study sites, ranging between four species at Barranco del Toril (Peña Negra) and Las Cruces 2 (Caurel) and 13 at Matabejid (Mágina) (Fig. 4 and Table 1; for a complete description of the ant assemblages see Manzaneda *et al.* 2007). More than half of these ant species were elaiosome consumers, but legitimate and facultative dispersers dominated quantitatively most disperser assemblages (Manzaneda *et al.* 2007). The ant species assemblage of hellebore

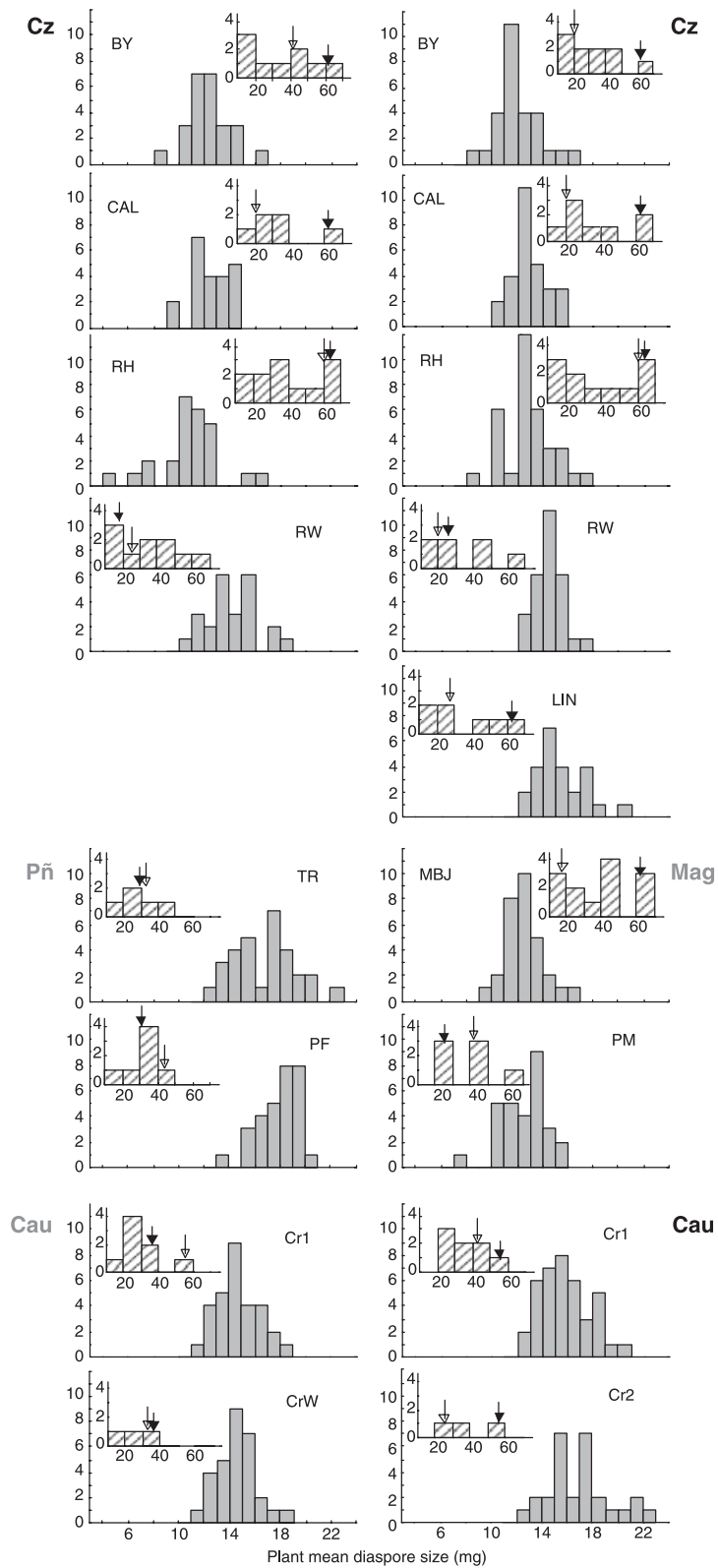


Fig. 4. Diaspore and ant species size distributions in the study populations in 2002 (left column of panels) and 2003 (right column). Data represent the number of plants with a particular mean diaspore size (major panels) and the number of ant species with a given body size (inner small panels) in each study population. To facilitate comparison similar axis scales are used for all populations. Arrows inside the ant size panels indicate sizes of the first (black-headed arrow) and the second (white-headed arrow) most frequent ant visitors in each population (see ant species identity in Table 1). Ant sizes come from Garrido *et al.* (2002) and our unpublished data, and indicate head width of the ant species in dissecting microscope micrometer units (1 micrometer unit = 0.03125 mm). Populations within a region are indicated with upper-case (abbreviations follow Table 1), while regions are indicated with lower-case (Cz = Cazorla, Mag = Mágina, Pñ = Peña Negra, Cau = Caurel). Regions with high abundance of legitimate dispersers (see text) are in bold type, while regions with low abundance of legitimate dispersers or high interference by elaiosome consumers are in grey type.

Table 2. Diaspore correlates of seed dispersal within each population and year. Standardized regression coefficients ($\beta \pm 1$ SE) are shown for elaiosome to seed ratio (E/S) and diaspore size (DS) which were estimated by bootstrapping. Coefficients significantly differing from zero are in bold type (marginally significant ones, $P < 0.1$, are marked with an asterisk). Abbreviations of population names follow Table 1.

	2002			2003		
	E/S	DS	R ²	E/S	DS	R ²
Cazorla						
BY	0.27 ± 0.25	-0.1 ± 0.25	0.05	-0.008 ± 0.20	0.14 ± 0.20	0.02
CAL	0.23 ± 0.19	0.8 ± 0.19	0.46	0.12 ± 0.19	0.18 ± 0.20	0.06
RH	0.13 ± 0.22	0.24 ± 0.22	0.05	0.44 ± 0.16	0.28 ± 0.16*	0.12
RW	0.50 ± 0.20	0.04 ± 0.20	0.16	-0.33 ± 0.18*	-0.22 ± 0.19	0.03
LIN	-	-	-	0.43 ± 0.20	0.07 ± 0.19	0.12
Mágina						
MBJ	-	-	-	0.05 ± 0.20	-0.36 ± 0.20*	0.05
PM	-	-	-	0.29 ± 0.18	-0.40 ± 0.18	0.15
Peña Negra						
TR	0.09 ± 0.21	-0.33 ± 0.21	0.02	-	-	-
PF	0.22 ± 0.20	-0.38 ± 0.20*	0.05	-	-	-
Caurel						
Cr1	0.09 ± 0.17	-0.54 ± 0.17	0.24	-0.08 ± 0.17	-0.17 ± 0.17	0.03
Cr2	-	-	-	0.34 ± 0.27	0.67 ± 0.27	0.11
CrW	-0.08 ± 0.19	0.19 ± 0.19	0.04	-	-	-

was mainly constituted by three genera, *Camponotus*, *Formica* and *Lasius* (Table 1), which accounted for c. 73% of the ant visits; however, the identity of the most frequent ant visitors and the body size distribution of the ant visitor species varied among localities (Table 1; Fig. 4). It was apparent that the identity of the most frequent visitors of hellebore differed considerably between regions but not between populations within region (Table 1). As a result, the potential dispersers of hellebore were more similar in size within a region than between regions (see arrows indicating sizes of the most frequent ants in Fig. 4). This was partly confirmed by a pattern of higher resemblance in the ASL-ant size between closer populations during the second year of study (Mantel test, $r = 0.41$, $P = 0.05$). In 2002, there was a similar, but not significant, pattern (Mantel test, $r = 0.1$, $P > 0.1$).

Mean ant abundance (average number of workers per census) around the plants was largely variable among populations and ranged between 0.26 and 5.40. Ant abundance was particularly low in Caurel 2002 (0.34 ± 0.56 , mean \pm 1SD), whereas it was high in Mágina (3.64 ± 3.71) and Cazorla (1.53 ± 2.26 and 1.79 ± 2.56 , in 2002 and 2003, respectively). Despite intermediate ant abundance, the abundance of legitimate dispersers in Peña Negra was extremely low (0.04 ± 0.10), because the most frequent ant species were elaiosome consumers or facultative dispersers. Elaiosome consumers were also abundant at Mágina.

DIASPORE-RELATED CORRELATES OF SEED DISPERSAL

Taken separately from other traits, the diaspore significantly influenced seed removal rate at some

populations in all regions and years (Table 2). DS apparently affected dispersal positively in some populations and negatively in others (e.g. Cazorla vs. Mágina, respectively). E/S ratio did not correlate significantly with removal rate in any population from Mágina, Peña Negra or Caurel. By contrast, its influence in Cazorla was always positive when statistically significant (Roblehondo forest in 2002; Roblehondo and Linarejos in 2003; see Table 2). The influence of the diaspore was not always consistent within region (Table 3). In 2002 DS affected removal rate in the populations from Cazorla and Caurel differently, but not in populations from Peña Negra; in 2003 DS affected removal rate differently only in the populations from Caurel. E/S ratio effect on dispersal never differed between populations within a region.

The inconsistent relationship between diaspore and dispersal was confirmed after controlling for geographical distance dependence. Thus, DS tended to be positively related to dispersal across populations in 2002 (partial Mantel test, $r = 0.34$, $P = 0.06$) but the relationship tended to be negative in 2003 (partial Mantel test, $r = -0.34$, $P = 0.08$). By contrast, E/S ratio seemed not to be related to dispersal across populations (partial Mantel test, $r = -0.18$, $P = 0.23$, in 2002; $r = -0.20$, $P = 0.1$, in 2003).

DIASPORE INFLUENCE ON DISPERSAL PERFORMANCE RELATIVE TO FECUNDITY AND SURROUNDINGS

As stated in the methods, a preliminary multigroup test in SEM indicated that populations of the same region shared a common structure of causal relationships. The tests for the equality of path coefficients

Table 3. ANCOVA exploring the variation between populations of each region in the diaspore traits influencing the individual seed removal rate in 2002 (a) and 2003 (b). The relevant effects in these analyses are the interactions population \times trait. Significant effects ($P < 0.05$) are in bold type.

(a) 2002	Cazorla			Peña Negra			Caurel		
	g.l.	$F_{(82)}$	P	g.l.	$F_{(54)}$	P	g.l.	$F_{(48)}$	P
Population	3	8.41	< 0.0001	1	0.89	0.35	1	4.17	0.04
Diaspore size (DS)	1	4.87	0.03	1	3.57	0.06	1	3.72	0.06
E/S	1	1.94	0.16	1	0.09	0.75	1	0.58	0.45
Population \times DS	3	10.33	< 0.0001	1	0.003	0.95	1	5.09	0.03
Population \times E/S	3	2.58	0.06	1	1.74	0.19	1	0.63	0.43

(b) 2003	Cazorla			Mágina			Caurel		
	g.l.	$F_{(128)}$	P	g.l.	$F_{(58)}$	P	g.l.	$F_{(49)}$	P
Population	4	0.35	0.84	1	1.08	0.30	1	3.92	0.05
Diaspore size (DS)	1	0.57	0.45	1	5.44	0.02	1	1.34	0.25
E/S	1	1.40	0.24	1	0.08	0.77	1	0.15	0.69
Population \times DS	4	0.49	0.74	1	0.09	0.76	1	5.76	0.019
Population \times E/S	4	1.60	0.18	1	1.02	0.32	1	0.17	0.68

between populations from a region (data available upon request) always showed significant differences in a minor number of paths from the measurement model (the part of the SEM not involving relationships between latent variables, see Fig. 2). In addition, except for a significant difference in Mágina on the path from the Plant factor to Performance, no significant difference between populations was found in the relationships between latent variables (the Structural model) within each region or year. Therefore, most paths or relationships can be assumed to be equal among populations of the same region. These results suggest that the model of total equality of path coefficients (i.e. some kind of average for each path between populations of the same region) adequately represents the intensity of the effects of the measured variables and factors (latent constructs) on the dispersal performance.

Assuming for each region the model of total equality of path coefficients, chi-square goodness of fit tests always indicated good model fits ($\chi^2 = 186.0$, d.f. = 206, $P = 0.89$; $\chi^2 = 235.5$, d.f. = 201, $P = 0.87$, for Cazorla in 2002 and 2003, respectively; $\chi^2 = 90.7$, d.f. = 96, $P = 0.63$, for Peña Negra; $\chi^2 = 88.4$, d.f. = 96, $P = 0.70$; $\chi^2 = 85.6$, d.f. = 96, $P = 0.76$, for Caurel in 2002 and 2003, respectively; $\chi^2 = 76.4$, d.f. = 96, $P = 0.93$ for Mágina). Furthermore, RMSEA values were always below the cut-off points used to accept a model as good or very good (RMSEA value < 0.1 or < 0.05 , respectively). Taken together, these fit tests indicate that the effects of Surroundings, Plant and Diaspore on early dispersal performance are adequately depicted under the hypothesis of the causal relationships of the model. The model explained more than 50% of variance in the individual dispersal performance in Cazorla in 2002 and 100% in 2003, 54% in Peña Negra, 95% in Caurel in 2002 and 20% in 2003, and 47% in Mágina.

The variation in individual dispersal performance in Cazorla in 2002 was influenced by diaspore and plant surroundings (Fig. 5), with a major effect of the former (as indicated by its higher path coefficient). Diaspore size and E/S ratio contributed to the diaspore effect negatively and positively, respectively. The surroundings of the plant affected dispersal through positive effects on ant abundance, proximity to other reproductive hellebores and interference by elaiosome consumption. Dispersal performance was affected in decreasing order of importance by diaspore and plant fecundity in 2003 (Fig. 5). Again, diaspore influence was due both to its size and to the E/S ratio, but this time both affected dispersal positively. Plant fecundity influenced dispersal positively through seed crop and number of seeds per carpel. Both diaspore and plant fecundity also had an indirect negative effect on dispersal through a trade-off (negative correlation).

In Peña Negra, variation in the plant surroundings and, to a lesser extent, in diaspores, influenced the individual variation in dispersal performance. Ant abundance contributed positively to the surrounding effect (Fig. 5). Diaspore affected dispersal through its size but, because the effect was negative, large diaspores were unfavourable to dispersal.

In Mágina, plant surroundings and plant fecundity influenced the individual variation in dispersal (Fig. 5). Plant fecundity affected dispersal through the positive effect of seed crop and seed number per carpel. The influence of the surroundings was negative and determined by ant abundance and elaiosome consumption: the higher the activity of the ants in the neighbourhood of a plant, the higher the interference by elaiosome consumers and the lower its seed removal.

Finally, dispersal was exclusively determined by plant surrounding features in Caurel in 2002, with ant abundance and interference contributing positively

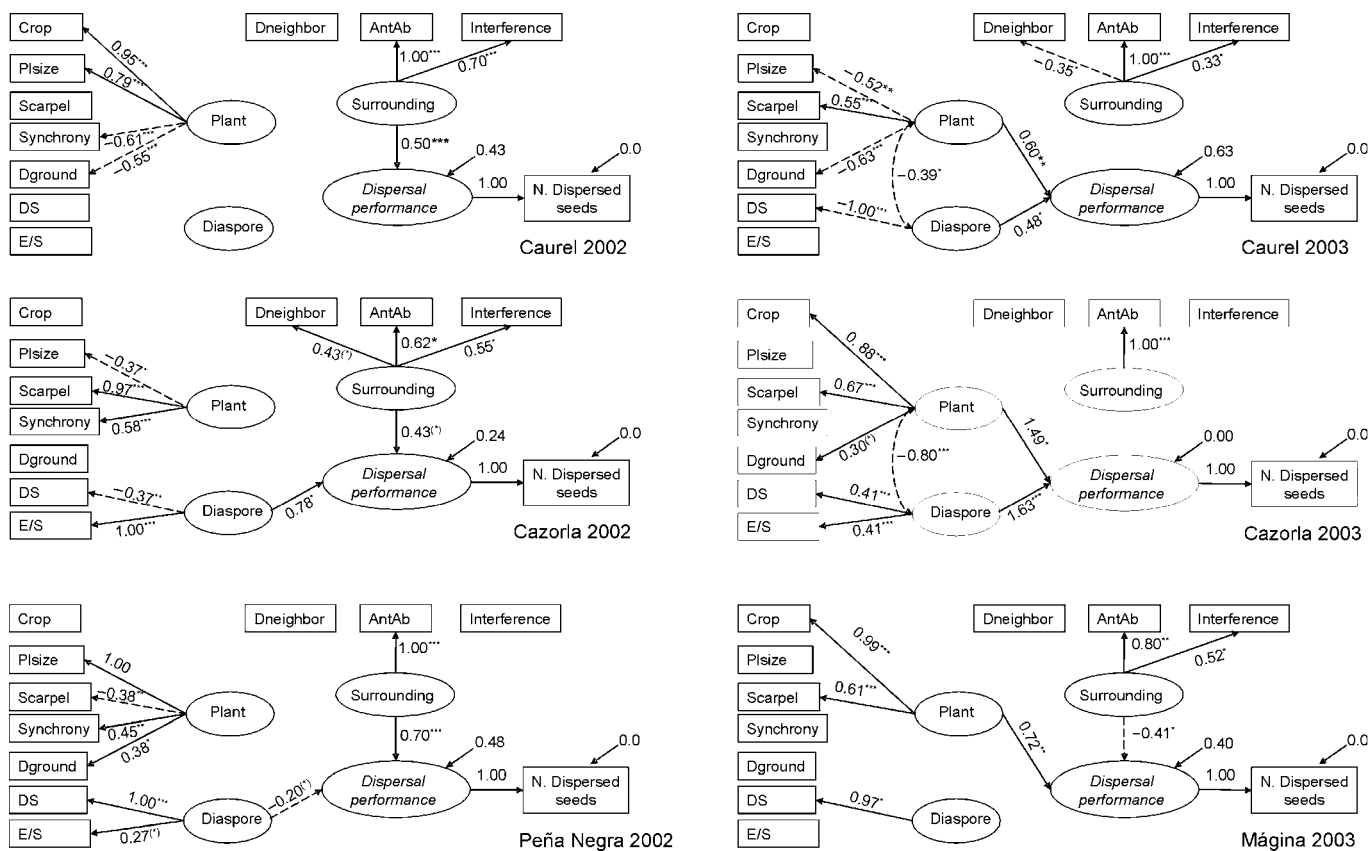


Fig. 5. Path diagrams for each region and year showing the influence of diaspore, plant fecundity and surrounding (latent construct variables indicated on ellipses) on the dispersal performance of hellebore. Only paths with coefficients differing from zero (coefficients on the paths arrows) are shown. Negative effects appear as dashed lines, positive ones as solid lines. (*) $P < 0.1$, ($*$) $P < 0.05$, ($**$) $P < 0.01$, ($***$) $P < 0.001$.

(Fig. 5). In 2003, the major dispersal determinants were plant fecundity and diaspore variation. Plant fecundity affected dispersal through the positive effect of the number of seeds per carpel and the negative effect of plant size and distance of carpels to the ground. Diaspore variation affected dispersal through the negative effect of its size (Fig. 5). However, plant fecundity and diaspore also influenced dispersal indirectly through a trade-off.

Discussion

The major objectives of this paper were to evaluate the contribution of diaspore to early dispersal performance relative to plant surroundings and fecundity, and to assess if large-scale (geographical) variation in ant disperser assemblages was related to a differential contribution to dispersal of these three groups of dispersal correlates. These objectives have been accomplished by path analyses, which have confirmed a major role of the diaspore in dispersal, alongside a pervasive influence of the surrounding of the plant. Diaspore effect on dispersal appeared in three out of four regions and in four out of six combinations of region and year. Nonetheless, the effect of diaspore was not geographically or temporally consistent. This was partly related to functional and compositional

differences in ant assemblages. As will be discussed, the appearance of an effect of seed traits on dispersal depended on the surroundings of the plant and on ant abundance.

GEOGRAPHICAL VARIATION IN THE DETERMINANTS OF SEED DISPERSAL

Exploration by path analyses showed geographical and temporal inconsistencies in the major determinants of the early individual dispersal performance. The most pervasive determinant (which appeared in all regions) was the ecological surroundings of each plant. Surroundings acted mainly through ant abundance which, except for Mágina, always had a positive net effect on dispersal (but not significant in Caurel or Cazorla in the second year). The close vicinity to each plant thus had a predictable effect on dispersal success. Additional data from the same populations of this study suggest that ant abundance, especially the abundance of legitimate ant dispersers, may limit seed dispersal in this species both on the finest scale (each plant) and on the local and geographical scale (Manzaneda *et al.* 2007). Other studies have shown that abundance of ant seed dispersers may limit dispersal of myrmecochorous plants (e.g. Christian 2001; Gómez *et al.* 2003). If individual dispersal performance

depends on ant abundance, then it would be expected that abundance in the close vicinity of each plant was the major determinant of seed removal in regions with low disperser abundance, while it should be unimportant in localities with high abundance of legitimate dispersers. This is again confirmed by our results. The region with highest scarcity of legitimate dispersers was Peña Negra, where the effect of the surroundings of each plant explained a major proportion of the variance in seed removal. Similarly, ant abundance in Caurel in 2002 was rather low, but it was the major (and the only significant) determinant of seed removal. In contrast, in Cazorla, where ants were abundant, the surroundings were less important (non-significant in 2003) and their effects were surpassed by the effect of diaspore traits.

The influence of diaspore on dispersal was also pervasive and it was frequently stronger (as estimated by the path coefficients) than the effect of plant fecundity or the surroundings. The effect of diaspore on dispersal varied geographically and temporally: sometimes it was neutral, sometimes positive or negative. In Mágina, the diaspore did not affect individual variation in dispersal, which was mainly affected by plant fecundity (through crop size) and surroundings, in this case through a negative influence of ant abundance and interference with elaiosome consumption (Fig. 5). The ant assemblage in Mágina populations was formed by small ants, which often consumed the elaiosome *in situ*. This is the case of *Formica cunicularia* and *Lasius niger*, which behave as facultative dispersers, or *Crematogaster sordidula*, *Tetramorium caespitum* and *Tapinoma nigerrimum*, which behave as elaiosome consumers (Manzaneda *et al.* 2007; Table 1). Elaiosome consumption caused high levels of interference with dispersal and ultimately resulted in a negative effect on dispersal. In contrast to Mágina, in Cazorla, the other southern locality, the diaspore was the main determinant of dispersal in both study years: we consistently found that the more rewarding the diaspores of a plant, the higher its seed removal. This suggests an optimization ant strategy based on achieving high elaiosome reward (high E/S ratio). This strategy has been described as the most common pattern of selection in myrmecochory (Hughes & Westoby 1992; Edwards *et al.* 2006). The influence of DS, however, was inconsistent, being negative in one year and positive in the other. Ant assemblage in this region was dominated by *Camponotus cruentatus* in most populations, a large ant able to carry seeds of very different sizes and which always acts as a legitimate disperser of hellebore (Manzaneda *et al.* 2007). In Peña Negra, the negative influence of DS and E/S on dispersal suggests that ants tended to avoid large diaspores (i.e. to avoid the burden of large seeds). Ant assemblage in this region was composed by small ants (mainly *Lasius emarginatus*), and the diaspores were the largest (Table 1 and Fig. 4). Finally, the diaspore in Caurel influenced dispersal only in 2003. Here, plants

with smaller diaspores obtained higher dispersal rates, indicating preference for plants with a small load to carry. Ant assemblages were dominated by *Formica lugubris* (a large ant able to carry any hellebore diaspore) and, to a lesser extent, by *Lasius fuliginosus*, a small ant able to recruit workers towards a food source. Diaspores in Caurel were large, particularly in 2003 (Table 1; Fig. 4). Assuming neutral choice by the large *F. lugubris*, it seems reasonable that the activity of the small *L. fuliginosus* primed plants with smaller seeds.

Although it did so less frequently, maternal fecundity also influenced dispersal success, both directly and indirectly through their influence on diaspore traits. A trade-off between seed size and seed number is assumed in many theoretical analyses (Smith & Fretwell 1974; Geritz 1995; see Garrido *et al.* 2005 in the case of hellebore), but it is not often extended to its consequences on the seed dispersal rate of individual plants (but see Smith *et al.* 1986; Ganeshaiah & Uma Shaanker 1991; Jordano 1995). We found this trade-off in two regions, Cazorla and Caurel in 2003. Plants with larger seed crops and seed number per carpel had smaller DS and E/S ratio in Cazorla. This reduced their dispersal success because it was favoured by larger and more rewarding diaspores. In Caurel, there was a trade-off between seed number per carpel and diaspore size, but the trade-off here had positive consequences because dispersal was higher in plants with small diaspores.

We have thus shown that there is both regional-scale geographical and temporal variation in dispersal-related diaspore traits and ant disperser assemblages in hellebore. The effects that this spatial and temporal variation had on hellebore seed removal were inconsistent. Diaspore size and its reward, and plant fecundity traits, only affected dispersal success if ants were abundant. Otherwise, ant abundance itself affected dispersal positively. Moreover, the plant or diaspore trait that significantly affected seed removal at any given time and in any given region depended on which ant disperser species were locally abundant. Unlike other studies that have demonstrated the influence of some traits (of seed, plant or surroundings) separately, this study highlights the need to consider all of these traits together in order to clarify the actual contribution of a trait to dispersal.

Concluding evolutionary remarks

Dispersal is a key plant life-history process probably affecting fitness. Traits involved in dispersal success are thus expected to be targets of selection (Herrera 2002). In elaiosome-bearing seeds, elaiosome and seed size, and the ratio between the two, are the most acknowledged targets of selection by ants (Edwards *et al.* 2006; Giladi 2006). Given that hellebore seeds are adapted to be dispersed by ants, selection on diaspore traits is likely to affect dispersal mediated by ant dispersal agents. Research on the dispersal system of hellebore in a macroecological scale has recently suggested that shifts

in ant disperser assemblages are responsible for spatial differences in the selection on diaspore size (Garrido *et al.* 2002; Alcántara *et al.* 2007) or elaiosome chemistry (Boulay *et al.* 2007). A limitation of these studies is that they did not consider other factors potentially influencing dispersal in the field and, as such, their value may be open to question. The geographical and temporal variations found here in the diaspore effects on dispersal point to the existence of differences in the selection on diaspore at dispersal. However, the finding of a significant effect of ant species assemblage on seed removal does not rule out many other sources of selection on diaspore traits, such as through trade-offs, maternal effects, and direct effects of the environment on seed dispersal, germination and establishment. We have also shown that such effects are interdependent in the dispersal stage: the appearance of an effect of the diaspore (or plant fecundity) on dispersal was dependent upon the surroundings of the plant, the identity of the ant visitors and on ant abundance. This dependence itself might lead to different directions of selection among populations. Such a conclusion is difficult to draw solely from observing diaspore offerings to ants, the usual protocol in studies on myrmecochory.

Acknowledgements

We are grateful to Beni, R. Boulay and J. Bastida for field assistance. We are indebted to C.M. Herrera and J. Guitián for their support during this work. We thank Salva Valera and K. Prasad for corrections to the English text. The Junta Rectora of Cazorla, Segura y las Villas and of Sierra de Mágina Natural Parks provided working facilities. Financial support came from Spanish M.E.C. Grant BOS2000-1122-C03-02, BOS2000-1122-C03-03 and BOS2003-03979-C02-02. While writing this paper, A.J.M. received funds from J. Manzaneda and A. Ávila.

References

- Alcántara, J.M., Rey, P.J., Manzaneda, A.J., Boulay, R., Ramírez, J.M. & Fedriani, J.M. (2007) Geographic variation in the adaptive landscape for seed size at dispersal in the myrmecochorous *Helleborus foetidus*. *Evolutionary Ecology*, **21**, 411–430.
- Alcántara, J.M., Rey, P.J., Valera, F. & Sánchez-Lafuente, A.M. (2000) Factors shaping the seedfall pattern of a bird-dispersed plant. *Ecology*, **81**, 1937–1950.
- Beattie, A.J. & Hughes, L. (2002) Ant–plant interactions. *Plant–Animal Interactions. An Evolutionary Approach* (eds C.M. Herrera & O. Pellmyr), pp. 211–235. Blackwell Science, Oxford.
- Bonnet, E. & Van der Peer, Y. (2002) Zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software*, **7**, 1–12.
- Boulay, R., Coll-Toledano, J., Manzaneda, A.J. & Cerda, X. (2007) Geographical variations in seed dispersal by ants: are plant and seed traits decisive? *Naturwissenschaften*, **94**, 242–246.
- Boulay, R., Fedriani, J.M., Manzaneda, A.J. & Cerda, X.

- (2005) Indirect effects of alternative food resources in an ant–plant interaction. *Oecologia*, **144**, 72–79.
- Boulay, R., Coll-Toledano, J. & Cerda, X. (2006) Geographic variations in *Helleborus foetidus* elaiosome lipids composition: implications for dispersal by ants. *Chemoecology*, **16**, 1–7.
- Caruso, C.M. (2000) Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution*, **54**, 1546–1557.
- Christian, C.E. (2001) Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature*, **413**, 635–639.
- Edwards, W., Dunlop, M. & Rodgerson, L. (2006) The evolution of rewards: seed dispersal, seed size and elaiosome size. *Journal of Ecology*, **94**, 687–694.
- Fedriani, J.M., Rey, P.J., Garrido, J.L., Guitián, J., Herrera, C.M., Medrano, M., Sánchez-Lafuente, A.M. & Cerda, X. (2004) Geographical variation in the potential of mice to constrain an ant–seed dispersal mutualism. *Oikos*, **105**, 181–191.
- Ganeshiaiah, L.N. & Uma Shaanker, R. (1991) Seed size optimization in a wind dispersed tree, *Butea monosperma*: a trade-off between seedling establishment and pod dispersal efficiency. *Oikos*, **60**, 3–6.
- Garrido, J.L., Rey, P.J., Cerda, X. & Herrera, C.M. (2002) Geographical variation in diaspore traits of an ant-dispersed plant (*Helleborus foetidus*): are ant community composition and diaspore traits correlated? *Journal of Ecology*, **90**, 446–455.
- Garrido, J.L., Rey, P.J. & Herrera, C.M. (2005) Pre- and post-germination determinants of spatial variation in recruitment in the perennial herb *Helleborus foetidus* L. (Ranunculaceae). *Journal of Ecology*, **93**, 60–66.
- Geritz, S.A.H. (1995) Evolutionary stable seed polymorphism and small-scale variation in seedling density. *American Naturalist*, **146**, 685–707.
- Giladi, I. (2006) Choosing benefits or partners: a review of the evidence for evolution of myrmecochory. *Oikos*, **112**, 481–492.
- Gómez, C., Pons, P. & Bas, J.M. (2003) Effects of the Argentine ant *Linepithema humile* on seed dispersal and seedling emergence of *Rhamnus alaternus*. *Ecography*, **26**, 532–538.
- Grace, J.B. (2003) Comparing groups using structural equations. *Structural Equation Modeling. Applications in Ecological and Evolutionary Biology* (eds B.H. Pugesek, A. Tomer & A. von Eye), pp. 281–296. Cambridge University Press, Cambridge.
- Herrera, C.M. (1993) Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecological Monographs*, **63**, 251–275.
- Herrera, C.M. (1995) Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology*, **76**, 1516–1524.
- Herrera, C.M. (2000) Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. *Ecology*, **81**, 2170–2176.
- Herrera, C.M. (2002) Seed dispersal by vertebrates. *Plant–Animal Interactions. An Evolutionary Approach* (eds C.M. Herrera & O. Pellmyr), pp. 185–210. Blackwell Science, Oxford.
- Herrera, C.M., Medrano, M., Rey, P.J., Sánchez-Lafuente, A.M., García, M.B., Guitián, J. & Manzaneda, A.J. (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences*, **99**, 16823–16828.
- Hughes, L. & Westoby, M. (1990) Removal rates of seeds adapted for dispersal by ants. *Ecology*, **71**, 138–148.
- Hughes, L. & Westoby, M. (1992) Effect of diaspore characteristics on removal of seeds adapted for dispersal by ants. *Ecology*, **73**, 1300–1312.

- Jordano, P. (1995) Frugivore-mediated selection on fruit and seed size: birds and St. Lucie's Cherry *Prunus mahaleb*. *Ecology*, **76**, 2627–2639.
- Kalish, S., Hanzawa, F.M., Tonsor, S.J., Thiede, D.A. & Voigt, S. (1999) Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology*, **80**, 2620–2634.
- Manly, B.F.J. (1997) *Randomization, Bootstrap, and Monte Carlo Methods in Biology*. Chapman & Hall, London.
- Manzaneda, A.J., Rey, P.J. & Boulay, R. (2007) Geographical and temporal variation in the ant-seed dispersal assemblage of the perennial herb *Helleborus foetidus* L. *Biological Journal of the Linnean Society*, **00**, 000–000.
- Mark, S. & Olesen, J.M. (1996) Importance of elaiosome to removal of ant-dispersed seeds. *Oecologia*, **107**, 95–101.
- Ness, J.H. & Bressmer, K. (2005) Abiotic influences on the behaviour of rodents, ants, and plants affect an ant-seed mutualism. *Ecoscience*, **12**, 76–81.
- Ohkawara, K., Higashi, S. & Ohara, M. (1996) Effects of ants, ground beetles and the seed-fall patterns on myrmecochory of *Erythronium japonicum* Decne. (Liliaceae). *Oecologia*, **106**, 500–506.
- Ohkawara, K., Ohara, M. & Higashi, S. (1997) The evolution of ant-dispersal in a spring-ephemeral *Corydalis ambigua* (Papaveraceae): timing of seed-fall and effects of ants and ground beetles. *Ecography*, **20**, 217–223.
- Oostermeijer, J.G.B. (1989) Myrmecochory in *Polygala vulgaris* L., *Luzula campestris* (L.) DC. and *Viola curtisii* Forster in a Dutch dune area. *Oecologia*, **78**, 302–311.
- Peters, M., Oberrath, R. & Böhning-Gaese, K. (2003) Seed dispersal by ants: are seed preferences influenced by foraging strategies or historical constraints? *Flora*, **198**, 413–420.
- Pugesek, B.H., Tomer, A. & von Eye, A. (2003) *Structural Equation Modeling. Applications in Ecological and Evolutionary Biology*. Cambridge University Press, Cambridge.
- Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Rey, P.J., Herrera, C.M., Guitián, J., Cerdá, X., Sánchez-Lafuente, A.M., Medrano, M. & Garrido, J.L. (2006) The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology*, **19**, 21–34.
- SAS Institute (1990) *SAS/STAT User's Guide*. SAS Institute Inc, Cary, NC.
- Scheiner, S.M., Mitchell, R.J. & Callahan, H.S. (2000) Using path analysis to measure natural selection. *Journal of Evolutionary Biology*, **13**, 423–433.
- Shipley, B. (1997) Exploratory path analysis with applications in ecology and evolution. *American Naturalist*, **149**, 1113–1148.
- Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *American Naturalist*, **108**, 499–506.
- Smith, B.H., Ronsheim, M.L. & Swartz, K.R. (1986) Reproductive ecology of *Jeffersonia diphylla* (Berberidaceae). *American Journal of Botany*, **73**, 1416–1426.
- StatSoft. (2000) *STATISTICA for Windows* [Computer Program Manual]. StatSoft, Tulsa, OK.
- Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multi-species plant–animal interactions. *Annual Review of Ecology, Evolution, and Systematic*, **35**, 435–466.
- Tomer, A. & Pugesek, B.H. (2003) Guidelines for the implementation and publication of structural equation models. *Structural Equation Modeling. Applications in Ecological and Evolutionary Biology* (eds B.H. Pugesek, A. Tomer & A. von Eye), pp. 125–140. Cambridge University Press, Cambridge.
- Werner, K. & Ebel, F. (1994) Zur Lebensgeschichte der Gattung *Helleborus* L. (Ranunculaceae). *Flora*, **189**, 97–130.

Received: 10 January 2007; accepted: 12 July 2007

Handling Editor: Peter Klinkhamer