



Geographic variation in seed removal of a myrmecochorous herb: influence of variation in functional guild and species composition of the disperser assemblage through spatial and temporal scales

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The ecological influence of changes in the functional guild and species composition of ant assemblages on ant-dispersal mutualisms is still poorly known. Using a multi-scale approach over an 800 km range within the Iberian Peninsula, we tested the hypothesis that variation in seed removal rate was related to functional guild rather than to species composition variations of disperser assemblages in the myrmecochore herb *Helleborus foetidus*. At least two premises must be confirmed to validate this hypothesis: 1) ant assemblages that are dissimilar in species composition but with similar functional guild composition will not differ significantly in seed removal, and 2) assemblages with different functional guild composition will render different seed removal services. We conducted 3328 ant-visitor censuses on 462 individual plants to identify both the species composition and functional guild variation of the ant-disperser assemblage, and the plant seed removal rate. Functional guild composition of the ant assemblage was determined by the proportion of visits of ants acting as legitimate dispersers, facultative dispersers or elaiosome predators. Results showed that ant-seed dispersal success seemed to be more sensitive to species composition changes of the ant assemblage than to functional guild shifts. However, this sensitivity was scale-dependent. Thus, at the fine, inter-individual scale, seed removal covaried with the species and functional guild composition of the ant assemblages; at the inter-population scale, differences in seed removal tended to be related to the dissimilarity of the assemblage species composition rather than the assemblage functional guild; finally, inter-regional differences in seed removal were unrelated to dissimilarities of the ant assemblage composition or functional guild. Though differences in seed removal and the relative frequency of the legitimate dispersers tended to be positively correlated, none of the above premises were fully confirmed in this study. Therefore, our results did not support in full the hypothesis that the variation in seed removal was explained by shifts in functional guild composition, rather than shifts in species composition.

Factors determining ecological patterns in nature frequently differ according to the scale considered (Blackburn and Gaston 2002). The importance of the scale is widely accepted in the study of communities or in macroecology (Böhning-Gaese 1997, Blackburn and Gaston 2002), but has received little attention in studies on plant-seed dispersal mutualisms (see, however, Rey 1995, Kollmann 2000, Burns 2004, García et al. 2005, Bråthen et al. 2007). Although evidence is still scarce, several studies suggest that the interpretation of the patterns and consequences of seed dispersal are scale-dependent. For example, in avian frugivory, a recent evaluation of the adaptive origin of bird-fruit abundance patterns has shown that the relative importance of adaptive versus nonadaptive processes strongly depends on the scale of resolution (i.e. global, regional, population, or temporal; Burns 2004). Similarly, the relative importance of key seed dispersal determinants, such as the structure of the habitat where the interaction

takes place or the disperser assemblage composition and/or abundance, also seems to vary according to the scale considered (Kollmann 2000, García et al. 2001). Finally, it has been recently suggested that some demographic effects of seed dispersal depend on the similarity in the scale to which the major variation on seed dispersal and seed predation occurs (García et al. 2005).

Quantifying how variation in the disperser assemblage composition relates to early seed dispersal success (i.e. seed removal rates) is an essential prerequisite to evaluate the demographic and evolutionary potential of seed dispersal mutualisms (Jordano and Schupp 2000). This is because variations in disperser assemblage composition influence variation in seed removal and in the specific arrival environment of the seeds, largely impinging on later components of the dispersal performance and individual fitness (i.e. escape from predation, germination and seedling establishment of dispersed seeds; Jordano and

Schupp 2000, Rey and Alcántara 2000, Alcántara et al. 2000, Traveset et al. 2003). Spatial and temporal variation in disperser assemblages may manifest itself in a variety of components like disperser abundance, species composition and/or functional guild composition. The latter is determined by the relative abundance of legitimate dispersers, facultative dispersers and reward consumers (non-dispersers) within a given assemblage (Jordano 1994, Jordano and Schupp 2000, Manzaneda et al. 2007). Therefore, the influence of disperser assemblage variation on the seed removal rate may depend on the assemblage component analyzed.

In the case of ant-dispersed plants, spatial and temporal variation in ant-disperser assemblages is well known. This variation occurs in the total number of ant species of the assemblage (Gómez et al. 2003), in the species composition (Gorb and Gorb 1999, Garrido et al. 2002), and in the functional guild composition (Cuautle et al. 2005, Manzaneda et al. 2007). This variation affects seed removal rates (Pudlo et al. 1980, Horvitz and Schemske 1986, Hughes and Westoby 1990, Gorb and Gorb 1999) and subsequent stages of the dispersal cycle (Manzaneda et al. 2005). Variation in ant visitor assemblages may yield concomitant variation in seed removal rates, but it is not known whether ant seed removal is more sensitive to functional guild or species composition changes of the ant assemblage. What is more, the extent of the congruency of this sensitivity over different scales is still unknown. Knowledge of the influence of functional guild versus compositional variation of the disperser assemblage will clarify whether the ecological consequence of ant-seed dispersal is attributable to the action of the whole ant-visitor assemblage or, if it is ascribable only to a particular ant species or group, within the ant-visitor assemblage.

This paper describes the seed removal rate in the myrmecochorus perennial herb *Helleborus foetidus* (Ranunculaceae) over a geographic range of 800 km across the Iberian Peninsula. Multiple scales are considered: between regions (inter-regional scale), populations of one and the same region (inter-population scale), individuals within population (inter-individual scale) and years (inter-annual scale). We also investigate the extent to which observed variations in ant seed removal are affected by functional guild versus species composition variations of the disperser assemblage. To our knowledge, this is the first attempt to relate variation in early seed dispersal success to shifts in the disperser assemblage in a multi-scale context for an ant-seed dispersal system. Previous research on this dispersal system has demonstrated that ant assemblages may vary noticeably along the distribution range, between different habitats and between years (Garrido et al. 2002, Manzaneda et al. 2007). The determinants of inter-individual variation in seed removal also vary geographically (Rey and Manzaneda 2007), partly due to differences in assemblage composition. The sensitivity of seed dispersal to shifts in the ant assemblages composition and functional guild composition was not explicitly examined in our previous research. It is here examined using a multi-scale approach. To this end, focus is on relative rather than absolute abundance of ant dispersers for two reasons: 1) it is more informative for comparison with previous studies on seed dispersal, which are based on relative abundance (for example, Jordano

1994); and 2) absolute abundance of legitimate dispersers is not informative for the abundance of reward consumers, which may interfere with seed dispersal (Rey and Manzaneda 2007). We also compare the extent of the correlation of seed removal with both absolute and relative abundance of dispersers.

Specifically, this paper tests the hypothesis that variation in seed removal results from spatial and temporal shifts in functional guild composition rather than shifts in species composition. This hypothesis is deeply rooted in community functionality beliefs, where different species within the assemblage would develop similar ecological functions (functional equivalence and/or redundancy, Petchey and Gaston 2002; see Zamora 2000 for animal-plant interactions). In this view, the shared function of the species of a guild (rather than the identity of the species) would be important for community function and, in this case, for the performance of the myrmecochory interaction. At least two premises must be confirmed to validate this hypothesis: 1) ant assemblages which are dissimilar in species composition but with similar functional guilds (with comparable relative frequency of each interactive guild) will not differ significantly in seed removal service; 2) assemblages with different functional guild composition will play different seed removal roles. Also, a significant relationship is expected to occur between differences in seed removal rates and variations in the relative frequency of legitimate dispersers, since their visits most likely result in seed removal events (Manzaneda et al. 2007).

Methods

Study system and sites

Helleborus foetidus (hereafter, hellebore) is a perennial herb distributed throughout central and southern western Europe. In the Iberian Peninsula, it typically grows at middle to high elevations in northern and south-eastern mountain areas, where it occupies patchy scrublands and the understory of deciduous and mixed forests. Plants produce one or several ramets, each of which yields one terminal inflorescence after several seasons of vegetative growth. Flowers have 1–6 carpels, each with 10–16 elaiosome-bearing seeds. The elaiosome is constituted mainly by oleic acid (Boulay et al. 2006). It is soft and white and comprises 3–15% of the diaspore fresh mass (total diaspore mass ranges between 5 and 23 mg). Fruit maturation and seed shedding take place in June–July. Ants are attracted by the elaiosome and either remove the diaspore after it has fallen to the ground or climb the plant to collect diaspores directly from dehiscing carpels. The diaspores are carried to the nests, where the elaiosome is removed and eaten, and the intact seed may be discarded within the nest or on a waste-midden on the surface. Other aspects of the dispersal ecology of hellebore can be found elsewhere (Garrido et al. 2002, Manzaneda et al. 2005, Alcántara et al. 2007).

This study was conducted on 10 populations at 4 separate regions in the Iberian peninsula in June–July from 2001 to 2003 (Table 1). Two regions, Cazorla and Mágina, are south-eastern Mediterranean mountain ranges, and they are 100 km apart. The third region, Peña Negra, is

Table 1. Study sites and inter-regional and inter-population variation within the Iberian peninsula in the dispersal behavior of the hellebore ant-visitor assemblage during the dispersal event of 2003. Values depict mean \pm 1 SE. LDs: legitimate dispersers, FDs: facultative dispersers, ECs: elaiosome consumers. Highest values in the relative frequency of each interactive behavior between regions and populations are shown in boldface type. See Manzaneda et al. (2007) for a complete description of the study sites.

Region	Population	Elevation (m a.s.l)	Geographical coordinates	Relative abundance (%) of each interactive behavior			
				LDs	FDs	ECs	
Mágina	Matabejid	1100	37°43'N, 3°30'W	61.27 \pm 6.39	8.51 \pm 3.78	30.21 \pm 6.05	
	Puerto de la Mata	1650	37°44'N, 3°28'W	15.62 \pm 8.80	34.37 \pm 11.02	50.00 \pm 11.28	
Cazorla	Barranco la Yedra	1200	37°58'N, 3°54'W	Average region	45.39 \pm 6.05	17.51 \pm 4.84	37.09 \pm 5.67
		1450	37°58'N, 3°53'W		33.71 \pm 8.47	31.48 \pm 9.17	34.79 \pm 8.14
	Roblehondo	1270	37°56'N, 3°52'W		63.89 \pm 8.11	20.09 \pm 6.33	16.00 \pm 5.54
	Roblehondo-Forest	1290	37°56'N, 3°52'W		74.21 \pm 8.25	13.09 \pm 6.82	12.69 \pm 6.18
	Linarejos	1290	37°56'N, 3°52'W		4.54 \pm 4.54	49.09 \pm 8.79	46.36 \pm 8.76
		1110	37°56'N, 3°55'W		58.48 \pm 11.08	25.63 \pm 10.27	15.88 \pm 8.27
Average region				46.56 \pm 4.36	27.98 \pm 3.81	25.44 \pm 3.51	
Peña Negra	Barranco del Toril	1430	40°26'N, 5°18'W		8.97 \pm 6.15	42.31 \pm 12.46	48.71 \pm 12.72
Caurel	Las Cruces 1	1500	42°36'N, 7°6'W		46.87 \pm 10.57	25.21 \pm 8.70	27.91 \pm 9.05
	Las Cruces 2	1500	42°36'N, 7°6'W	Average region	88.89 \pm 7.62	3.70 \pm 3.70	7.41 \pm 7.62
					66.77 \pm 7.39	15.02 \pm 5.15	18.20 \pm 5.67

a central Mediterranean mountain system >400 km north of the former two. The last region, Caurel, is a north-western mountain range of the Eurosiberian domain, 800 km north of the southern regions and 400 km north-west of the central region of Peña Negra. These populations cover much of the natural range of hellebore in the Iberian Peninsula. They represent the diverse ecological conditions in which this species occurs (Table 1) as well as the various ant communities with which this species interacts.

Ant-visitor assemblages and functional guilds

Our data were obtained by conducting ant-visitor censuses on individual hellebore adults during the seed release period (June–July). In each population and year, 12–45 reproductive plants were selected and between 6 and 22 censuses were carried out per plant (3624 censuses on 486 plants, in total; see Manzaneda et al. 2007 for details on the sampling effort). Each census recorded every ant taxon visiting the diaspores, the number of individuals of each ant taxon visiting the plant, and the number of diaspores removed by each individual ant in a five minute period. The interactive behaviors shown by each ant species were classified according to their frequency (i.e. frequency of interaction sensu Vázquez et al. 2005) as one of three functional groups of interactive behavior (i.e. functional guilds of the ant-disperser assemblage): 1) legitimate dispersers (LDs) are ant species which act mainly as seed removers (i.e. taking away the entire diaspore from the parent plant and relocating it into the ant nest); 2) elaiosome consumers (ECs) are ant species that feed on elaiosome not dispersing the seed; and 3) facultative dispersers (FDs) are ant species which act indistinctly (i.e. with a roughly similar frequency) as legitimate dispersers or as elaiosome consumers. The interactive behavior displayed by the various ant species of the hellebore ant-visitor assemblage is described in detail in Manzaneda et al. (2007).

We recorded 35 ant taxa visiting hellebore diaspores in our study areas in the period 2001–2003, ranging between 4 species at Barranco del Toril and Las Cruces 2 and 13 species at Matabejid. Most of these ant species act as ECs.

Yet, ant species of the genera *Camponotus* and *Formica*, which frequently act as LDs, dominate quantitatively the ant-visitor assemblage in most of our study populations. The relative frequency of ECs, LDs and FDs within the ant-visitor assemblage varies notably at the inter-regional, inter-population (Table 1) and inter-individual scale (Supplementary material). Inter-annual variation in the relative abundance of these three functional guilds is less pronounced (Supplementary material and Manzaneda et al. 2007).

Individual seed removal measurement at multiple scales

Seed removal rate was calculated in each ant-visitor census and for each individual plant as the number of removed seeds divided by the number of seeds available per plant in the census. The number of available seeds in each census was obtained by summing the total number of seeds on the ground and the number of seeds in dehisced carpels on the plant.

Data from 2002 and 2003 (3328 censuses on 462 individual plants in 10 populations with a total sampling time of 16640 min of observation) were used to document seed removal patterns at all four scales (inter-regional, inter-population, inter-individual, and inter-annual scales).

Inter-population and inter-regional variations in seed removal and their correlation with the ant assemblage were analyzed in 2003 (26–45 plants per population, with 1993 censuses on 309 reproductive plants). Inter-individual variation in ant seed removal is shown for one population, Matabejid. This population was selected for its high ant activity on the hellebore plants, and was studied in June 2003 (Table 1). In this case, data were collected from 30 plants, with a total of 366 censuses. The longest distance between plants was 150 m and the shortest 1 m.

Finally, inter-annual variation in seed removal rates was examined over a 3-yr period (2001–2003) in two populations of Cazorla: Barranco de la Yedra and Calvario (Table 1). In this case, 12–26 plants were chosen in each population and 1027 censuses were conducted.

Data analysis

The relative magnitude of inter-region, inter-population, inter-individual and inter-annual variation in seed removal was quantified with a hierarchical variance partitioning analysis using the Covtest statement in the Mixed procedure of SAS (SAS 2005). In this analysis, all hierarchical levels were considered as random effects. The model was fitted with restricted maximum likelihood to account for the intrinsic lack of balance of this design (Quinn and Keough 2002). In addition to the statistical significance of variance components, variation in seed removal in each scale was also studied fitting separately generalized linear models and generalized linear mixed models (Genmod and Glimmix Procedure in SAS, respectively). The seed removal rates were modeled with binomial distribution, using logit as link function. Thus, a model (with Region as fixed factor and Population nested within Region as random factor) was used for significant inter-regional and inter-population variations in seed removal. The inter-individual variation in seed removal within the Matabejid population was analyzed with a model considering Plant as a fixed factor. The inter-annual variation in seed removal by ants in two populations within Cazorla was analyzed fitting two separate models, one per population, with year as fixed factor.

Partial Mantel's tests for the inter-regional, inter-population, inter-individual and inter-annual scales of resolution were conducted separately to identify whether differences in seed removal were related to differences in the ant-visitor species (assemblage species composition, Supplementary material) or to differences in ant interactive behavior (functional guild composition). This test allows, through a permutation procedure, to analyze the degree of correlation between three distance matrices through the Partial Mantel's statistic, r_M , which estimates the correlation between two of the matrices controlling the effect of the third (Casgrain and Legendre 2001). The matrices used are: 1) Euclidean distance matrix of the seed removal rates, formed by pairwise distances in seed removal rate; 2) distance matrix of the assemblage species composition, built by pairwise dissimilarity coefficients (1-PS); and 3) dissimilarity matrix of the assemblage of the functional guilds, built by pairwise dissimilarity coefficients (1-PS) in the relative abundance of the three groups of interactive behavior. Details of the matrices are given in Supplementary material. PS coefficients (proportional similarity Renkonen index) were obtained from the following formula:

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

where, for n species, p_{ai} is the relative abundance (estimated from frequency of interaction rather than from absolute or ambient abundance, Vázquez et al. 2005) of species i in a region, population, plant or year a , and p_{bi} is the relative abundance of species i in a region, population, plant or year b . The PS index ranges from 0 (standing for maximum dissimilarity, i.e. no common taxa between a and b) to 1 (standing for maximum resemblance, i.e. identical species composition between a and b). We tested the null hypothesis of no association between the matrix elements

in each pair of matrices (Manly 1991). Partial Mantel's statistic r_M for each analysis was computed after 10 000 permutations performed with the Mantel test module implemented in R Package v.4.6 program (Casgrain and Legendre 2001).

Spearman partial linear correlation analyses were used for the relationship between the variation in individual seed removal and the relative abundance of LDs controlling for the relative abundance effect of the rest of the interactive guilds of the assemblage. Separate partial correlations were used for each scale. All correlations were performed using the procedure CORR in SAS.

Finally, to test if shifts in the functional guilds composition of the ant-visitor assemblage rather than shifts in the species composition control the variation in seed removal at the population level, we use ternary graphs and posthoc comparisons (least significant difference tests, performed with SAS). The test compared seed removal rates between those pairs of populations with different species composition but with similar functional guild composition (four pairs of populations compared, see Supplementary material and Table 1 for details). We also test whether seed removal rates differ significantly between pairs of populations with different functional guild composition (six pairs of populations compared, Supplementary material, Table 1).

Results

Patterns of variation in seed removal

Spatial variations accounted for 97% of the variance in seed removal, while inter-annual variation accounted for only 3% (Table 2). Nonetheless, only the inter-individual variance, which explained most of the variation in seed removal (87%), differed significantly from zero (Table 2).

Detailed analysis on each scale shows that, overall, the seed removal rate per plant was low (mean \pm 1 SE: 5.06% \pm 2.14, $n=462$) and differed significantly at the inter-regional ($F_{3,299}=3.09$, $p=0.027$) and inter-population scales ($Z=12.23$, $p<0.0001$). In Cazorla, seed removal was significantly higher than in the other regions (Fig. 1A). By contrast, at Peña Negra ants removed far fewer seeds from the plants. Differences in seed removal were observed at the inter-population scale (Fig. 1B). Seed removal varied significantly at the inter-individual scale

Table 2. Result of the variance partition to analyze the inter-regional, inter-population, inter-individual, and inter-annual variation on seed removal rate (data of 2002 and 2003 on 462 individual plants across 10 populations, see Methods for details). Components of variance come from a hierarchical design with all levels considered as random factors. Percentages of variance differing significantly from zero are shown in boldface type.

	Significance		Variance (%)
	Z	p	
Region	0.733	0.463	5.2
Population	0.893	0.371	4.6
Plant	15.29	<0.0001	87.1
Year	0.882	0.377	3.1

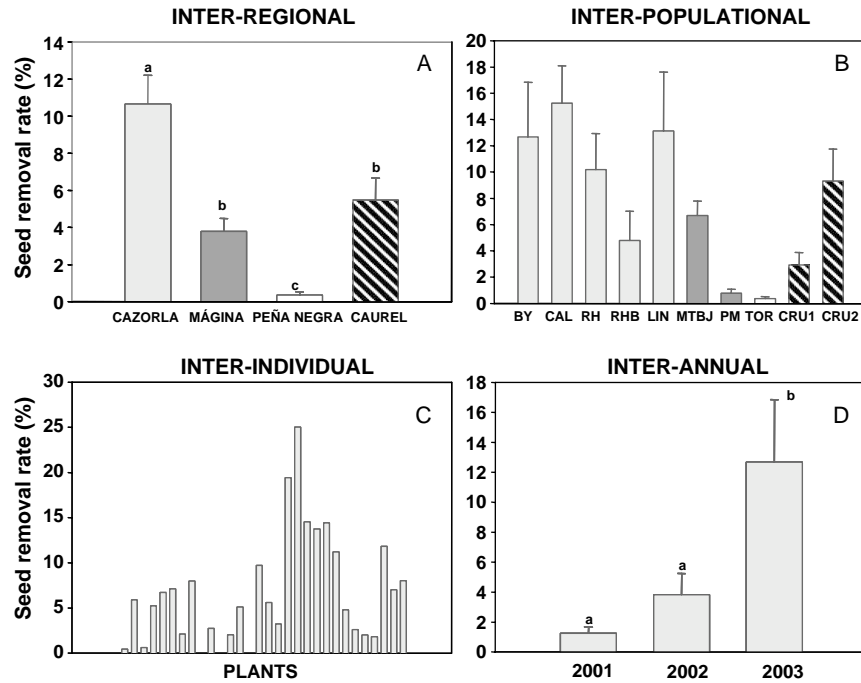


Figure 1. Pattern of variation in the individual seed removal rates of hellebore at multiple scales. Observed mean values ± 1 SE are shown in all cases except for inter-individual variation. When present, similar letters depict non-significant differences at $p = 0.05$ in pairwise posthoc tests. From left to right in Fig. 2B: Barranco de la Yedra (BY), Calvario (CAL), Roblehondo (RH), Roblehondo-Forest (RHB), Linarejos (LIN); Matabejid (MTBJ), Puerto de la Mata (PM); Barranco del Toril (Tor); Las Cruces-1 (Cru1), Las Cruces-2 (Cru2). Data for inter-individual and inter-annual variation come from MTBJ and BY, respectively.

(Wald $\chi^2 = 68.49$, $p < 0.0001$). This indicates that, as expected from the variance partitioning, seed removal differed substantially at the finest spatial scale. Thus, seed removal varied 25-fold between plants separated only by a few meters (Fig. 1C). Finally, seed removal differed significantly at the inter-annual scale in Barranco de la Yedra (Wald $\chi^2 = 247.23$, $p = 0.0035$), where seed removal was significantly higher in 2003 than in preceding years (Fig. 1D), but not in Calvario (Wald $\chi^2 = 2.38$, $p = 0.304$).

Ant-disperser assemblages and seed removal at different scales

At the inter-regional scale, dissimilarities in seed removal were not related significantly to the dissimilarities of the assemblage species composition ($r_M = -0.0247$, $p = 0.469$) or functional guild composition ($r_M = -0.0456$, $p = 0.517$). By contrast, at the inter-population scale, differences in seed removal tended to be related to the dissimilarity of the assemblage species composition ($r_M = 0.18$, $p = 0.114$) rather than to the functional guild composition ($r_M = -0.0182$, $p = 0.515$). After controlling for geographical distance (i.e. adding the matrix of pairwise geographic distances to the partial Mantel test, see also Supplementary material), a positive and significant correlation with seed removal was found for the assemblage composition ($r_M = 0.324$, $p = 0.021$), but not for the functional guild composition ($r_M = 0.028$, $p = 0.345$). Therefore, the more similar the ant-visitor assemblage is between populations, the more similar their mean seed removal (Fig. 2). By contrast, differences in seed removal rates between pairs of populations were constantly variable

over the range of dissimilarity in functional guild composition (Fig. 2).

At the inter-individual scale, differences in seed removal rates between plants were positive and significantly related both to the dissimilarity of the ant assemblage composition ($r_M = 0.177$, $p = 0.031$) and to the dissimilarity in the functional guild composition ($r_M = 0.291$, $p = 0.002$). This suggests that seed removal covaried with the species composition and functional guilds of the ant assemblages at fine scales.

Finally, in the population with significant inter-annual variation in seed removal (Barranco de la Yedra, see above), no significant relationship was found between variation in the species composition or functional guild composition of the assemblage and the inter-annual shifts in the seed removal rates ($r_M = 0.4217$, $p = 0.331$; $r_M = 0.053$, $p = 0.541$ for assemblage composition and functional guild composition, respectively).

Legitimate disperser abundance and seed removal

Seed removal tended to be positively related at the inter-regional scale to variations in the relative abundance of LDs when controlling for the effects of the relative abundance of ECs and FDs. Still, this relationship did not reach statistical significance (Spearman partial correlation coefficient, $r_s = 0.608$, $p = 0.391$, $n = 4$; Fig. 3) due to the low number of regions analyzed. Similarly, at the inter-population level, an increase in the average relative abundance of LDs corresponded to a marginally significant increase in seed removal rates ($r_s = 0.63$, $p = 0.068$, $n = 10$; Fig. 3). At the inter-individual scale, the mean relative abundance of LDs

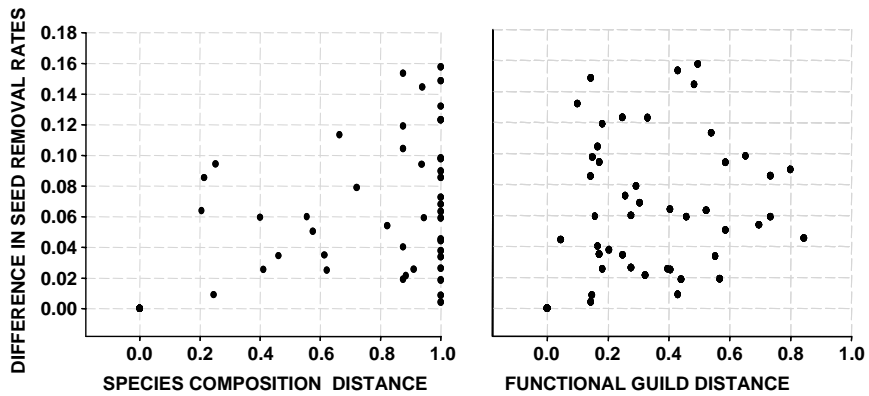


Figure 2. Left panel. Pairwise differences in seed removal rates between populations are depicted versus pairwise differences in dissimilarity coefficients (1-PS) of assemblage composition. Right panel. Pairwise differences in seed removal rates between populations are plotted versus pairwise dissimilarity coefficients (1-PS) in the functional guild composition (i.e. functional guild distance, estimated from the relative abundance of the three groups of interactive behavior).

correlated significantly with individual seed removal rates ($r_s = 0.69$, $p < 0.0001$, $n = 30$; Fig. 3). All these correlations became smaller and often non-significant when considering absolute instead of relative ant abundances ($r_s = 0.41$, $p = 0.73$, $n = 4$ at inter-regional scale; $r_s = 0.52$, $p = 0.18$, $n = 10$ at inter-population scale; $r_s = 0.62$,

$p < 0.001$, $n = 30$ at inter-individual scale). Moreover, relative abundance of LDs remained highly correlated with seed removal at most levels when controlling for absolute abundance (inter-regional scale: $r_s = 0.80$, $p = 0.40$, $n = 4$; inter-population scale: $r_s = 0.48$, $p = 0.18$, $n = 10$; inter-individual scale: $r_s = 0.65$, $p < 0.0001$, $n = 30$).

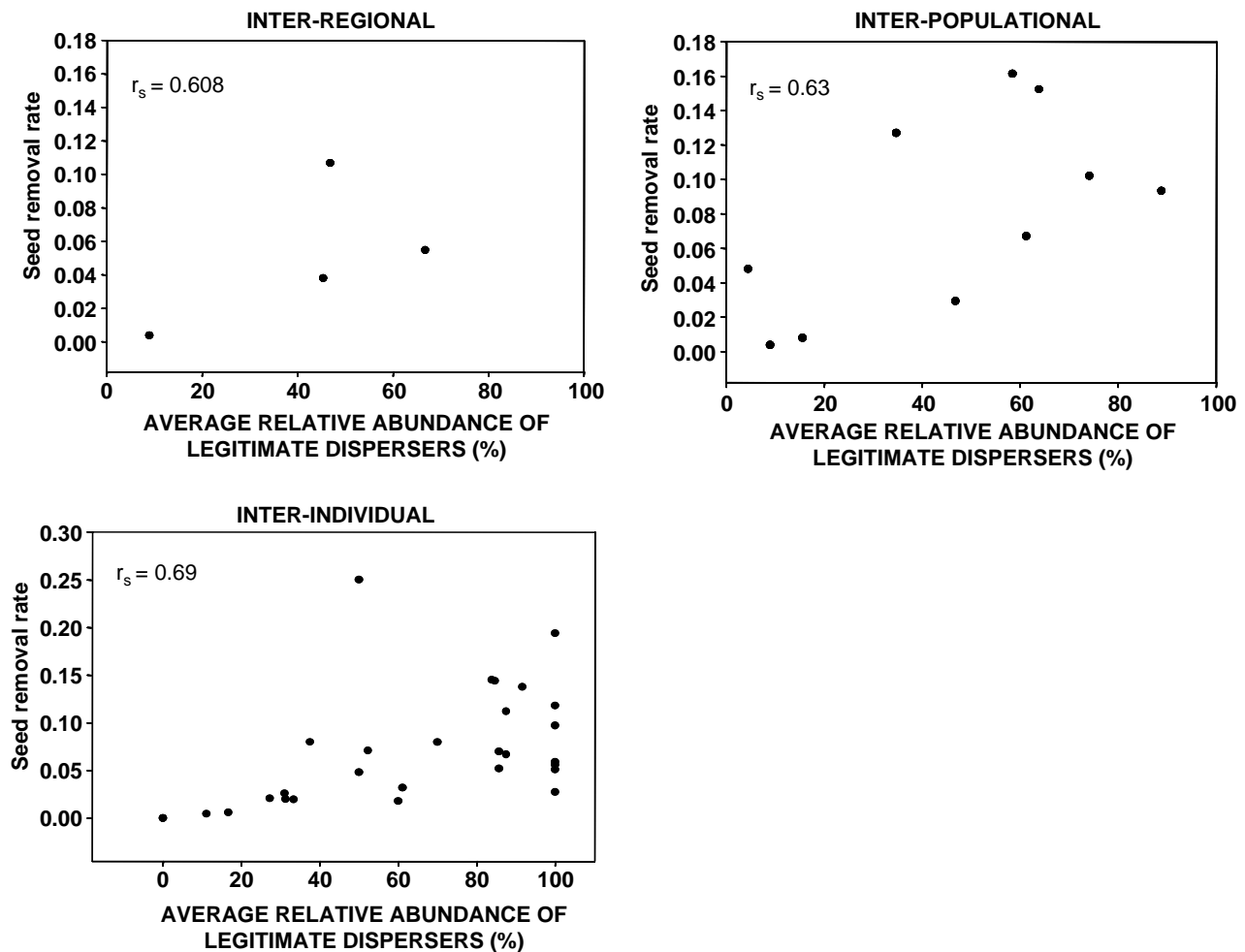


Figure 3. Relationship at multiple spatial scales between variation in the relative frequency of legitimate dispersers and individual variation in seed removal rate.

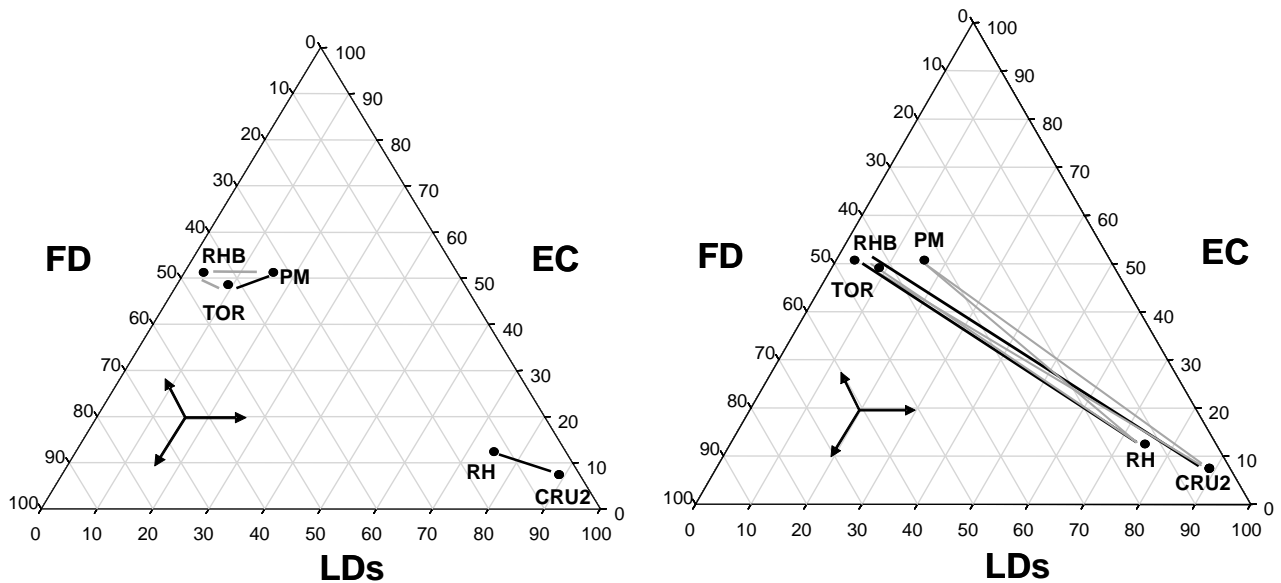


Figure 4. Ternary graphs showing the variation across the Iberian Peninsula in the functional guild composition of hellebore's ant assemblage and the similarity in seed removal rates: between 4 pairs of populations with different species but similar functional guild composition (left), and between 6 pairs of populations with very dissimilar functional guild composition (right). Data for functional guild composition come from Table 1. Clusters mean a similar functional guild composition of the assemblage. Black lines link populations with non-significant differences in seed removal rate (posthoc comparisons, $p = 0.05$); grey lines indicate significant differences in seed removal rate between populations. Acronyms of populations as in Fig. 1. LDs: legitimate dispersers, FD: facultative dispersers, EC: eliasome consumers. Arrows in the graph indicate how to read the three coordinates of a point on the ternary graph (note that the sum of the three coordinates is always 100).

However, the correlation of absolute abundance of LDs with seed removal was non-significant (inter-regional scale: $r_s = -0.22$, $p = 0.86$, $n = 4$; inter-population scale: $r_s = -0.19$, $p = 0.63$, $n = 10$; inter-individual scale: $r_s = 0.37$, $p = 0.05$, $n = 30$). Finally, inter-annual shifts in seed removal in Barranco de la Yedra was unrelated to inter-annual shifts in the relative abundance of LDs ($r_s = 0.2$, $p = 0.608$, $n = 3$).

Functional equivalence between populations

Ternary graphs and posthoc comparisons showed that only 2 out of 4 comparisons between populations that showed similar functional guild composition but different in species composition yielded similar significant seed removal rates (Fig. 4). Likewise, posthocs conducted between populations with dissimilar functional guild composition showed that seed removal differed significantly in 4 out of 6 comparisons (Fig. 4).

Discussion

Early seed dispersal success is known to vary considerably in space and time. This paper shows that this seems to apply in myrmecochory systems too. We tested the hypothesis that the variation is explained in terms of shifts in the functional guild composition of the disperser assemblage rather than shifts in its species composition. Although differences in seed removal rates and in the relative frequency of the LDs tended to be positively correlated, our results do not support this hypothesis completely, because some predic-

tions which are necessary to confirm it were not fully supported: 1) ant assemblages with similar functional guild but different in species composition showed dissimilar seed removal rates at most scales; 2) assemblages with different functional guild composition frequently played similar seed removal roles at inter-population (Fig. 4) and inter-individual scales. Our results suggest that, overall, seed removal seems to be more sensitive to changes in the ant assemblage species composition than to functional guild shifts, but also that this sensitivity is scale-dependent. It may be argued that the hypothesis of functional equivalence should be examined based on absolute rather than relative abundance of the ant visitor functional guilds. However, our results showed that seed removal was more sensitive to variation in relative than absolute abundance of LDs in all three spatial levels. This justifies our focus on relative abundances.

Spatial and temporal variation in seed removal

Seed removal was more variable in space than in time. In particular, most variance was due to inter-individual differences in seed removal, that is, to fine-scale variation. Seed removal also varied at the inter-regional, inter-population and, at least in one of the populations, at the inter-annual scale. This pattern of variation across scales agrees with other results of myrmecochorous plants, which also described remarkable variation in seed removal rates, especially, at fine spatial scales (Horvitz and Schemske 1986, Smith et al. 1989, Hughes and Westoby 1990, Guitián et al. 2003). Therefore, fine-scale variation of early seed dispersal success seems to be the rule in this type of

mutualism, and is probably concomitant to the scale at which ants forage (Horvitz and Le Corff 1993). The fact that major variation in seed removal occurred at the inter-individual scale suggests an opportunity for natural selection to occur at the seed dispersal stage, whenever differences in dispersal success result in differences in seedling establishment.

Ant-disperser assemblages and seed removal covariation at multiple scales

The influence (or not) of functional guild or species composition shifts of the ant assemblages on seed removal in hellebore depends on the scale considered. At the inter-individual scale, shifts in the functional guild composition explained more variation in seed removal between individuals than shifts in assemblage composition (29.06 versus 17.7%, see Results). In other words, a substantial fraction of the variation in seed removal was due to the fine-scale differential occurrence of different guilds of interactive behavior at the inter-individual scale. Specifically, the importance of the functional guild composition for individual seed dispersal success was closely linked to the importance of the relative frequency of the LDs within its assemblage: the higher the representation of the LDs in the visitor assemblage of a plant, the higher the individual seed dispersal success (Fig. 3). However, unlike other scale levels, the inter-individual variation in dispersal success, which is the most relevant scale for micro-evolutionary processes, depends both on functional guild and species composition components of the assemblage. This means that some functional equivalence of the ant species is probably relevant to the early seed dispersal success of each plant, but also that differences in dispersal success will emerge due to the identity of the ant species visiting each plant. For example, the interactive behaviour of each ant-visitor with the seeds is influenced primarily by the ratio of diaspore size to ant size (Manzaneda et al. 2007), and the probability to be dispersed is highly correlated to ant size (Garrido et al. 2002). We would thus expect that, even within the guild of the LDs, the larger the ant species is the better its dispersal service in terms of seed removal. In fact, large formicines, like *Camponotus cruentatus* or *C. vagus* (in southern populations) or *Formica lugubris* (in northern ones) remove the vast majority of hellebore diaspores (Manzaneda et al. 2007, Manzaneda and Rey unpubl.). We also expect the species composition component of the ant assemblage to increase in ecological relevance in later stages of the plant recruitment as a result of ant species-specific differences in the fate of the seeds in nests (Manzaneda et al. 2005, Giladi 2006).

At larger spatial scales, variations in the functional guild composition did not explain differences in seed removal. Seed removal tended to be positively related to the relative frequency of LDs across populations, but inter-population differences in seed removal were largely related to differences in ant assemblage composition (Fig. 2). Thus, macroecological differences in seed dispersal success seems to be influenced by assemblage composition, which in turn is influenced by habitat type and structure, geographic distance (Garrido et al. 2002, Manzaneda et al. 2007 and

references therein) and biogeography (i.e. differences between ant assemblages from Mediterranean and Euro-siberian domains). Research on hellebore has also shown that other components of the ant assemblage, such as total ant abundance, absolute abundance of LDs, and ant species-specific functional traits (e.g. ant size), influence seed dispersal success and may influence each assemblage's phenotypic selection on seed traits at the dispersal stage (Rey and Manzaneda 2007, Alcántara et al. 2007). This underlines the relevance of both species composition and functional guild components of the disperser assemblage in macro-ecological and micro-evolutionary terms.

Crossing across scales, spatial structuring of ant assemblages and disperser service

Our results suggest a spatial structuring of the early seed dispersal success in hellebore. Such a spatial structuring is mainly shaped at inter-individual scales, but at inter-population ones too, and results from a similar across-scale spatial pattern of ant-disperser assemblages (Manzaneda et al. 2007). These spatial structures may have implications for the evolution of the interaction. If the spatial structure of the seed removal pattern is not substantially altered in later stages of the dispersal cycle (i.e. through seed predation, germination and/or seedling establishment), it might result in a complex scenario of spatially variable selection. This scenario would be defined by: 1) differences between hellebore populations across the Iberian Peninsula in disperser assemblage composition (which is influenced mainly by habitat and biogeography); and 2) inter-individual differences in how the disperser assemblage of each plant is composed of the ants available in the local ant community (which is influenced by fine-scale topography, neighborhood effects and by individual plant traits, Rey and Manzaneda 2007). Under this complex spatial scenario, it would be possible to find local adaptive responses of the plants with seeds adjusted to the assemblage species composition (see also Alcántara et al. 2007). Yet, inter-individual variations of fine scale (micro-site level) in the functional guild composition, especially in the guild of LDs, may disrupt the overall selective pressure at the population and thus be an ecological constraint on the possibility of local adaptive responses of the plants.

The spatial structuring found here may not apply in all myrmecochory interactions, but the structuring of seed removal success can be expected to depend frequently on the spatial structure of the ant assemblage (see also Horvitz and Schemske 1990). Notably, the spatial structuring of this myrmecochory system differs from other seed dispersal systems mediated by animals (e.g. vertebrate frugivory), where fine-scale variations in seed removal seem to be less relevant (Kollmann 2000).

To conclude, our results underscore the importance of a multi-scale view in seed dispersal studies as some of the most important consequences for this type of interaction are usually scale-dependent (Kollmann 2000, Burns 2004, García et al. 2005, Bräthen et al. 2007). Failure to adopt a multi-scale approach may lead to an inaccurate understanding of the ecological and evolutionary significance of this animal-plant relationship.

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