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SHORT COMMUNICATION

Geographic variations in seed dispersal by ants: are plant and seed traits decisive?

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Abstract The effect of local ant species on the dispersal success of a myrmecochorous plant, *Helleborus foetidus*, was analyzed in two populations of the Iberian Peninsula (Caurel and Cazorla, respectively). The contribution of the various local ant species to dispersal was very unequal. While 5 and 19 ant taxa visited the plants of Caurel and Cazorla, respectively, most removal activity (67 and 80%) was performed by two species only (*Formica lugubris* and *Camponotus cruentatus*, respectively). Visits by dispersers were also unequally distributed between neighboring plants. While some plants were always visited during the period of seed release, others were never visited. A regression model indicated that this pattern might be explained by two plant traits: ants preferred to visit plants that released more seeds and whose elaiosomes were richer in oleic acid. Although it has long been known that this compound triggers removal by ants, it is the first demonstration that quantitative variations in elaiosome traits contribute to variation in dispersal success. Finally, other variables being equal, morphological traits (seed size, elaiosome size, and elaiosome/seed size ratio) did not affect ant behavior. Although myrmecochory has long been considered a diffuse interaction, our results support the idea that, at local scale, a limited number of ant species may be decisive to its evolution.

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Introduction

A major concern in evolutionary science is the evolution of mutualisms between free-living organisms (as opposed to parasites) involving many unspecialized partners that exert incongruent selection forces on each other. Because the outcome of such mutualisms may be difficult to predict, the role of reciprocal selection in their evolution is sometimes underestimated. However, if the interacting species are structured geographically, coevolution can act at the population level on a reduced number of partners, allowing local coadaptations (Thompson 2005). Such "diffuse" mutualisms are well exemplified by seed-ant dispersal interactions (myrmecochory) in which seeds bearing a lipid-rich appendage (the elaiosome) can be transported by large guilds of nongranivorous ants. More than 3,000 myrmecochores are known around the world, mainly in temperate and Mediterranean regions (Beattie and Hughes 2002) where ant communities can change rapidly along latitudinal gradients (Cushman et al. 1993; Retana and Cerdá 2000). As a consequence, across their range, myrmecochores are likely to interact with a variety of dispersers which have varying impacts on their reproductive success (Gorb and Gorb 1999; Peters et al. 2003).

According to Schupp (1993) and Jordano and Schupp (2000), the contribution of a disperser to a plant reproductive success depends on: (1) the number of seeds that leave the mother plant due to the disperser activity and (2) how the disperser manipulates the seeds during transport. The present study focuses on the first component, which is generally a function of the number of visits to the plant and of the number of seeds removed per visit. However, unlike birds or bats for which this framework was initially developed, ants rarely remove more than one seed at each visit. Therefore, the number of seeds that leave a plant due to the activity of an ant species mostly depends on the number of visits that the ant makes to the plant.

In theory, the number of seeds removed by a disperser can vary as a function of intrinsic plant factors (Jordano and Schupp 2000). Several studies have shown that the workers of an ant species may select between plant species as a function of diaspore morphology, rewarding quality, or plant crop size (review in Mayer et al. 2005). Nevertheless, how these factors affect ant choice at an intraspecific level is still elusive. For example, we only know of one study (Mark and Olesen 1996) that examines ant (*Myrmica ruginodis*) choice for different diaspore sizes of a plant (*Hepatica nobilis*). The result suggests that, at least in lab conditions, ants prefer diaspores with relatively large elaiosomes. A large body of evidence also indicates that ant retrieval behavior is triggered by elaiosome fatty acids. In particular, oleic acid, either free or as diolein, stimulates removal (Marshall et al. 1979; Skidmore and Heithaus 1988; Brew et al. 1989; Hughes et al. 1994; Boulay et al. 2006). However, it is not known whether natural variations in elaiosome oleic acid concentration affect ant visits in the field. Finally, Gorb and Gorb (2000) suggest that ant visits may depend on the density of seeds on the ground, but this result obtained with artificial seed depots awaits confirmation in real conditions.

The aim of the present study was to analyze geographic variations in the outcome of a myrmecochory. We analyzed the contribution of local ant species to the dispersal success of a myrmecochore, *Helleborus foetidus*, in two Iberian populations, one located in a Mediterranean and another in temperate region. We addressed the following issues: (1) What proportion of ants visiting hellebore shoots disperse their seeds? (2) Do disperser visits differ principally between individual plants, between groups of plants, or between populations? and (3) Can ant disperser visits to individual plants be predicted by seed mass, elaiosome mass, elaiosome/seed mass ratio, elaiosome oleic acid content, and diaspore releasing rate?

Materials and methods

Study system and field site

Details on the biology and ecology of *H. foetidus* are given elsewhere (e.g., Garrido et al. 2002). In general, reproductive shoots are composed of one to three 1-meter tall stalks bearing 25–100 fruits that release 30–40 diaspores each. Fieldwork was conducted in June and July 2003 in two populations of *H. foetidus* of the Iberian Peninsula separated by 650 km. The population of Caurel is located in northwest Spain (42°39′ N, 7°7′ W) and is characterized by a temperate climate. The vegetation consists of an open scrubland bordered by a *Pinus sylvestris* forest. The second population, Cazorla, is located in southern Spain (37°56′ N, 2°5′ W) and has a Mediterranean climate. In this region, plants grow in open fields with scarce trees (*Pinus nigra* and *Quercus rotundifolia*) and shrubs (mostly *Quercus ilex*, *Juniperus oxycedrus*, and *Rubus ulmifolius*).

Disperser censuses

Five and 6 groups (blocks hereafter) of nine plants were chosen in Caurel and Cazorla, respectively. The distance between neighboring plants in a block was about 1–2 m, while the distance between blocks in a population was at least 20 m. Over the period of carpel maturation the plants were monitored during 5-min long censuses during which the presence of ants on or below the plant was recorded. We classified ant behavior as either diaspore removal if a clear transport was observed or exploration for any other behavior. Hereafter, we define dispersers as any species that removed at least one seed throughout the censuses. Each plant was surveyed once a day during 5 to 12 consecutive days. In total, 698 censuses were done and on average, each plant was observed during a total of 35 min. The censuses were distributed between 9 a.m. (2 h after sunrise) and 9 p.m. (sunset) but the order in which the plants were surveyed changed every day. Thus, each plant was surveyed in various conditions of temperature, humidity, ant activity, etc. Although we did not survey the plants overnight, unpublished observations indicate diaspore transport by nocturnal ants such as *C. pilicornis* is very rare.

Diaspore weight and chemical analyses

Samples of recently released diaspores were collected during the censuses and kept at ambient temperature at most 6 h before being transferred to −20°C. Once in the laboratory, the elaiosomes and seeds from 410 diaspores (four to five diaspores per plant) were carefully detached and kept 72 h at ambient temperature. Their semidry weight was then measured to the nearest 0.1 mg. Chemical analyses were conducted on an independent set of 413 elaiosomes (two to seven elaiosomes per plant). The details of lipid extraction and analysis are presented by Boulay et al. (2006). After transesterification of the di- and triglycerides and methylation of the free fatty acids, the samples were analyzed by gas chromatography and flame ionization detection.

Data analysis

The contribution of each ant species to seed dispersal was estimated as the number of times a species was observed removing a diaspore divided by the total number of removal acts in the population. We compared the amount of the total variance in the number of visits of dispersers (irrespective of the species) explained by differences between populations, between blocks, between plants, and within plants (between censuses) using the variance component module of Statistica v.6 (Statsoft 2001) and the restricted maximum likelihood method. Finally, to test whether disperser visits were related to plant traits, we built a generalized linear model with the Poisson distribution and log link function. The response variable was the probability of visit by dispersers on a plant *i*, calculated as the proportion of censuses made to *i* in which at least one disperser (irrespective of the species) was observed. The continuous predictors were average seed mass, elaiosome mass, elaiosome/seed mass ratio, oleic acid content, and diaspore releasing rate of plant *i* plus the five second order interactions between the previous factors and population. Diaspore-releasing rate was calculated as:

$$\mathrm{DRR} = \frac{(\mathrm{OC_l} - \mathrm{OC_f}) \times \mathrm{NS}}{\mathrm{ND}}$$

where OC_f and OC_l are the numbers of open carpels on the first and last census, respectively, NS is the average number of seeds per carpel, and ND is the number of days between the first and the last census.

Results

The assemblages of *H. foetidus* seed dispersers differed widely between Cazorla and Caurel (Fig. 1). Only one species, *Lasius niger*, was observed in both populations. Within-populations disperser guilds did not differ substantially between blocks (data not shown). More species were observed on or beneath hellebore shoots in Cazorla (14 taxa) than in Caurel (five taxa). In both populations, the contribution of each species to dispersal was clearly uneven (Fig. 1). In Caurel *Formica lugubris*, a typical species from temperate European regions was responsible for 67% of the visits and 80% of the removals. In Cazorla, *Camponotus cruentatus* was responsible for 45% of the visits and 68% of the removals.

The probability of visits by dispersers differed mostly between plants within blocks and populations (Fig. 2). Hence, up to 55% of the total variance in the probability of visit by dispersers was explained by differences between neighboring plants (z=6.06, P<0.001). This suggested that some plants could be frequently visited while others located in the same block were rarely visited. In Cazorla, although all 54 plants released seeds during the period of observation, 18 and 16 plants were never and always visited by dispersers, respectively. In Caurel, 19 and 11 out of 45 plants were never and always visited, respectively. Differences within plants also explained a significant (z=17.22, P<0.001) but much lower (35%) amount of the total variance. Neither the population nor the block within the population explained a significant amount of variance in disperser visits (z=0.04 P=0.97 and z=0.94 P=0.34, respectively).

Whether a plant was frequently visited by dispersers or not could be predicted by two traits: the average amount of oleic acid contained in the elaiosomes and the rate at which diaspores were released (Table 1). For example, in Cazorla, the plants that were always visited released 106±13 seeds per day (mean±SD; n=18) and their elaiosomes contained 143±32 μg of oleic acid. By comparison, the plants that were never visited released only 61±10 seeds per day (n=16) and their elaiosomes contained 114±20 μg of oleic acid. In Caurel, the difference was even higher because always and never visited plants released 123±12 (n=19) and 65±13 (n=11) seeds per day, respectively, and their elaiosomes contained 173±49 and 121±20 μg of oleic acid, respectively. The other traits (seed and elaiosome mass and elaiosome/seed mass ratio) were not significantly associated with dispersers' visits. No second order interaction was significant, indicating that the effects of elaiosome oleic acid content and diaspore-releasing rate were similar in both populations.

Discussion

The first finding of the present study is that in two populations of *H. foetidus* separated by a large distance and inhabited by widely different ant species, most dispersal activity is dominated by two ant species (*F. lugubris* and *C. cruentatus* in Caurel and Cazorla, respectively). These major dispersers share several common features, such as large body-size, numerous and crowded nests, and long daily range of activity which make them major resource competitors and plant partners. Although many other species can react positively to the presentation of *H. foetidus* elaiosomes (Garrido et al. 2002), the censuses indicate that they rarely visit plants and may have only marginal effects on their reproductive success.

A second important finding is that, locally, plants are unevenly visited by dispersers as exemplified by the large amount of variance in disperser visits explained by differences between neighboring plants. In each block, several plants were never visited, while others were always visited by ants, irrespective of day and hour. How could this pattern be explained? Both *C. cruentatus* and *F. lugubris*

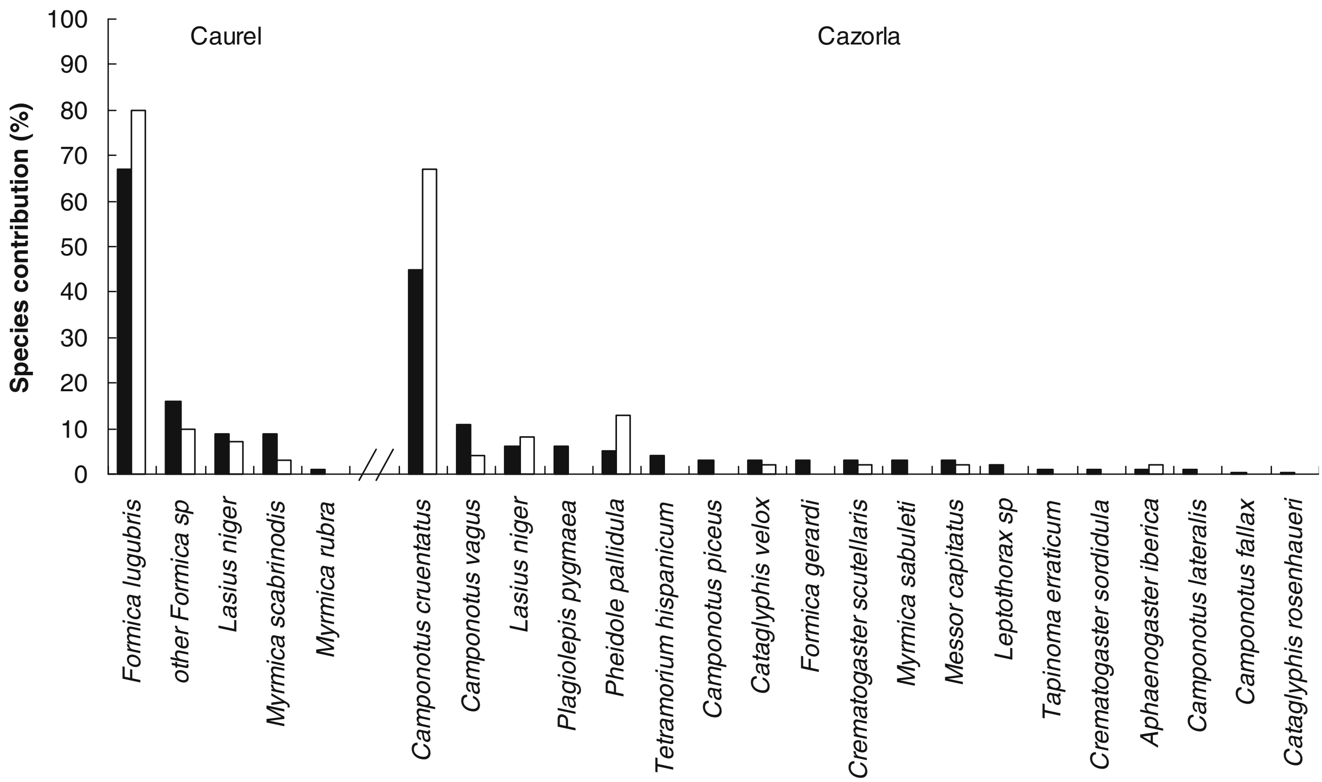

Fig. 1 Contribution (*percentages*) of the various ant species to *H. foetidus* visits (*black*) and diaspore removals (*white*) in Cazorla and Caurel. In Caurel, the group of *Formica* species was composed of *F. cunicularia* and *F. rufibarbis*. In Cazorla, the group of *Leptothorax* was composed of *L. unifasciatus*, *L. pardoi*, and *L. racovitzae*

generally forage over quite large distances (>30 m, personal observation) and should have been able to visit all the plants composing a block, independently of their exact location with respect to the nest. A recruitment process by which ants communicate the location of a food source to their nestmates might have generated recurrent ant visits to particular plants and the rejection of others. The result of the regression analysis suggests that other traits being equal dispersers were more likely to visit plants releasing many diaspores and whose elaiosomes were richer in oleic acid. This confirms previous results indicating that diaspore aggregation enhances removal rate (Gorb and Gorb 2000) and suggests that plants producing many seeds or releasing them during a limited time window may favor greater dispersal success. Moreover, it has long been known that oleic acid either in its free form or as a glyceride stimulates removal behavior (Marshall et al. 1979; Skidmore and Heithaus 1988; Brew et al. 1989; Hughes et al. 1994;

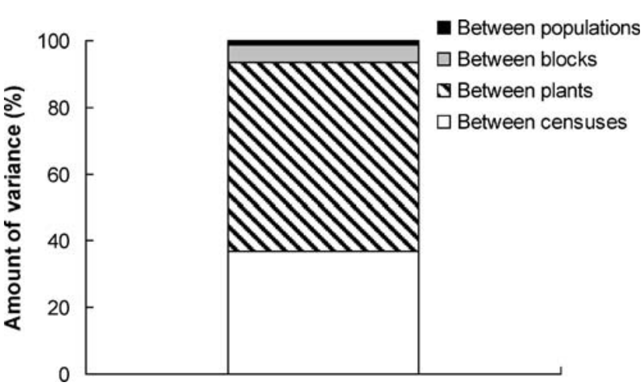

Fig. 2 Proportion of total variance in ant visits explained by differences between populations, between blocks within populations, between plants within blocks, and between censuses (within plants). Note that the variance explained by differences between populations (1.13%) is represented by the upper black line

Table 1 Results of the generalized linear model fitting the frequency of visits by dispersers to plant and diaspore traits and second order interactions with population

Variables	Chi-square	*P*
Elaiosome mass	0.02	0.8928
Seed mass	0.10	0.7526
Oleic acid content	14.89	<0.0001
Diaspore releasing rate	8.88	0.0029
Elaiosome/seed ratio	0.18	0.6684
Elaiosome mass × population	0.03	0.8557
Seed mass × population	0.33	0.5681
Oleic acid content × population	1.21	0.2716
Diaspore releasing rate × population	1.94	0.1632
Elaiosome/seed ratio × population	0.42	0.5162

Df=1 for all the variables

Boulay et al. 2006). Yet, this is the first time that intraspecific variations in oleic acid content have been related to ant service in the field. This fatty acid may provide a valuable energy supply for ant larvae (Fischer et al. 2005) that enhances colony fitness (Morales and Heithaus 1998; Bono and Heithaus 2002; Gammans et al. 2005). Nevertheless, our result is only correlative and other elaiosome components (sugars, vitamins...) that would covary with oleic acid might also be decisive in the choice of ants. By contrast, none of the three morphological traits studied was significantly related to ant visits. At an interspecific level, these traits limit dispersal by tiny ants that are not able to transport very large seeds (Mayer et al. 2005). However, intraspecific variations in diaspore shape or size may be too small to determine ant choice.

Myrmecochory is generally considered as a good example of diffuse mutualism in which plant dispersal depends on a gallery of nonobligate ant partners. This traditional view might be reconsidered because, at a local scale, the number of ant species that remove plant seeds is quite limited. The reason why only a small fraction of the ant community removes most of the seeds is intriguing and may be of particular importance for our understanding of the evolution of this interaction (Giladi 2006). Both *C. cruentatus* and *F. lugubris* selected the same plant and diaspore traits. Further studies are now needed to compare their effect on the overall plant fitness.

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