

Conflicting selection on diaspore traits limits the evolutionary potential of seed dispersal by ants

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Keywords:

ants;
directed dispersal;
multiplicative fitness components;
myrmecochory;
phenotypic selection;
seedling establishment.

Abstract

Conflicts of selection on diaspore traits throughout the dispersal cycle can limit the evolutionary consequences of seed dispersal. However, these conflicts have never been investigated in directed dispersal systems. We explored conflicts of selection through life stages of dispersal in the myrmecochorous herb *Helleborus foetidus*. Seeds are subject to two contrasting partial selective scenarios. Undispersed seeds are subject to positive directional selection on seed size characters, whereas seeds dispersed are subject to stabilizing selection for size. In both scenarios, seedling establishment determined the magnitude and direction of selection. This does not reflect ant preferences for seed size. However, total selection still depends largely on ant activity, as ants control the relative importance of each selective scenario. We advocate the use of analytical approaches combining multiplicative fitness and microenvironment-specific selection to more realistically estimate the realized selection on traits functional during several life stages. This approach may be extended to any organism dispersing offspring to different environments.

Introduction

There is growing evidence that the evolutionary potential of mutualistic plant–animal interactions may be limited by conflicting selective pressures through the plant life cycle (Campbell, 1991; Alcántara & Rey, 2003; Gómez, 2004, 2008). Such conflicts of selection appear when the effect of a phenotypic trait on one fitness component is modified or cancelled by its opposing effect on other fitness components (Schluter *et al.*, 1991). This is particularly relevant for mutualistic interactions occurring early in the plant life cycle, such as pollination and seed dispersal, because, apart from the possibility of opposing simultaneous interactions in such stages (Herrera *et al.*, 2002; Strauss *et al.*, 2005), their ecological consequences may be influenced by the sequential transitions throughout later life stages. Under such a view, transitions between life stages may be interpreted as distinct fitness

components (Crone, 2001). An appropriate analysis of plant fitness would thus require knowledge of multiple sequential stages and processes involved in the individual life cycle (Campbell, 1991; Gómez, 2008), and the net result of selection on a trait could then be understood by determining the influence of that trait on the multiplicative outcome of the different fitness components.

Seed dispersal is a key ecological process that connects seed production to the establishment of new offspring (Wang & Smith, 2002). Its consequences may thus be investigated by considering seed dispersal as one part within the recruitment phase of the life cycle. The recruitment phase involves different life stages, which proceed sequentially through different processes, such as seed removal from the mother plant, dispersal to a given microhabitat, survival until germination, seedling emergence and establishment (Herrera *et al.*, 1994; Jordano, 1995; Kollmann, 2000; Rey & Alcántara, 2000). In animal-dispersed plants, processes affecting seed and seedling performance can be spatially uncoupled (i.e. the best microhabitat for a seed may not be the best for a seedling), so conflicts of selection between dispersal and post-dispersal life stages can be expected (e.g. Herrera *et al.*, 1994; Schupp, 1995; Rey & Alcántara, 2000; Roth & Vander Wall, 2005). The evolutionary potential of seed

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dispersal mutualisms depends on whether selection exerted by dispersers extends beyond dispersal (up to seedling establishment or later) or whether such selection is modified by post-dispersal selective episodes. Recently, several studies have demonstrated the existence of conflicts of selection on dispersal-related diaspore traits (Alcántara & Rey, 2003; Martínez *et al.*, 2007).

Phenotypic selection on dispersal-related traits has rarely been studied in ant-dispersed plants (see Hughes & Westoby, 1992; Mark & Olesen, 1996; Boulay *et al.*, 2007) and no study has explored selection through multiple stages of the dispersal cycle. However, myrmecochory (i.e. seed dispersal by ants) provides an exceptional setting to explore selection through multiple stages as (1) seed deposition is highly directional, i.e. it is much more spatially predictable than many other dispersal systems (Wenny, 2001), and (2) it is possible to precisely evaluate seed removal rates from individual plants and the final fate for most of the seeds at the specific arrival sites, which is extremely difficult in other dispersal systems. Seeds dispersed by ants escape seed predation around the maternal parent and show enhanced seedling recruitment because of nutrient enrichment in ant nests (see reviews in Beattie & Hughes, 2002; Giladi, 2006). These adaptive advantages of myrmecochory occur at different stages of the dispersal cycle and facilitate the study of conflicting selection.

This study explores whether conflicts of selection among life stages limit the adaptive response of diaspore traits to selection imposed by ant seed dispersers. We develop a novel analytical approach to assess selection, taking advantage of the precise determination of seed arrival possible in directed dispersal systems. This approach combines multiplicative fitness components and microenvironment-specific selection analyses to more realistically estimate the realized selection on traits that are ecologically important in several life stages.

The size of the ant reward (elaiosome or other edible appendages attached to the seed), seed size and the ratio between the two are known targets of selection by ant dispersers (Edwards *et al.*, 2006; Giladi, 2006). Although other important traits like elaiosome chemistry seem to be under selection by ants (Boulay *et al.*, 2006, 2007), the effect of diaspore size extends beyond the seed removal phase to seed predation, germination and seedling survival (Moles & Westoby, 2006). Consequently, size-related diaspore traits are particularly relevant for examining conflicts of selection (Alcántara & Rey, 2003; Gómez, 2004) and we will focus here on these traits, using the myrmecochorous herb *Helleborus foetidus* L. (Ranunculaceae) as our study system. We evaluate the phenotypic selection exerted at dispersal by local ant disperser assemblages, further exploring the selection on the same trait in successive post-dispersal stages of the dispersal cycle. We combine field observations on indi-

vidual seed removal rates and arrival at ant nests with experimental trials that compare early seedling recruitment in ant nests with that from seeds remaining undispersed under the mother plant. We address the following questions: (1) Do ants exert phenotypic selection on size-related diaspore traits? (2) Do diaspore traits influence maternal fitness differently beneath the maternal plant (undispersed diaspores) and in ant nests (dispersed diaspores)? (3) Does phenotypic selection exerted by ants translate to higher seedling establishment or, on the contrary, is selection at dispersal cancelled or reversed by selection on subsequent stages? Our methodological approach allows us to explore the evolutionary consequences of possible changes in the ecological scenario. Specifically, we assess whether the pattern of selection on seed size would change in the absence of dispersers or if seedling mortality were 100% under the mother plants.

Methods

Study system and study area

The hellebore (*H. foetidus*) is a perennial herb distributed throughout central and southern Western Europe. It typically grows at middle to high elevations in mountains, occupying patchy scrublands and the understorey of deciduous and mixed forests. Flowers have one to six carpels, each containing 10–16 elaiosome-bearing seeds. The elaiosome, mainly constituted by oleic acid (Boulay *et al.*, 2006), accounts for 3–15% of the diaspore fresh mass (total diaspore mass ranges between 5 and 23 mg). Fruit maturation and seed shedding happen in June–July. Ants remove diaspores quickly and carry them to their nest. The intact seeds are then discarded within the nest or on waste-middens. Rodents (mainly wood mice, *Apodemus sylvaticus*) are important seed predators, and may consume seeds directly from the plant before ant activity (Fedriani *et al.*, 2004) or later at ant nests (Manzaneda *et al.*, 2005a). Ant nests of main dispersers, however, have been shown to be safe microhabitats for diaspores (Manzaneda *et al.*, 2005a). The ant visitor assemblages of the hellebore in the Iberian Peninsula have been described elsewhere (Garrido *et al.*, 2002; Manzaneda *et al.*, 2007). They are diverse both in species composition, size and dispersal behaviour and vary spatially at fine and large scales (Manzaneda *et al.*, 2007). In spite of the diversity of the ant visitor assemblages, most dispersal is carried out by just one or two species in many localities, making the system specialized at each site (Manzaneda & Rey, unpublished). Nests of some disperser ants are also nutrient-enriched microhabitats (Manzaneda, 2005). Most seeds germinate during the second spring (February–May) after entering the seed bank (Garrido *et al.*, 2005a). Many seedlings die after the first summer because of water stress (Manzaneda *et al.*, 2005b), but those that pass this first dry period

have a great probability to recruit and establish (Garrido *et al.*, 2005a).

This study was conducted from June 2002 to October 2004 near Roblehondo Field Station at Sierra de Cazorla (southeast of Spain, 37°56'N 3°52'W; 1300 m elevation). The study area is an evergreen oak–pine mixed forest. Hellebore populations in the area comprise only from several tens to few hundreds of reproductive individuals. The climate is Mediterranean, with a dry period typically extending from June to October. The average annual rainfall in the study area during the study period was 674 mm and the mean annual temperature was 11.8 °C.

Diaspore traits and seed crop size

The traits considered in phenotypic selection analyses were seed size and elaiosome size. We collected 5–10 dehiscent fruits from 73 individuals. We estimated seed crop size for each individual plant by counting the number of fruits and estimating from the collected fruits the mean number of seeds per fruit. Five to 12 seeds per fruit ($n = 2752$ diaspores) were weighed individually using a Mettler Toledo® xs105 precision scale. Seed size was estimated by the fresh mass of the diaspore once the elaiosome was removed, and elaiosome size was estimated by the difference between diaspore fresh mass and seed fresh mass. We then obtained individual plant means for seed and elaiosome mass to conduct the selection analyses.

Fitness components

We defined multiplicative fitness components of an individual as the probabilities of (1) diaspore removal by seed dispersers (W_r), (2) escaping from post-dispersal seed predators (W_p), (3) germination and emergence (W_g) and (4) surviving the first summer after germination (W_s). All of these fitness components were calculated for each mother plant as the ratio of the number of offspring completing a given stage to the number of offspring entering that stage.

Dispersal stage

To estimate W_r , we used data from ant visitor censuses on the mother plants during the seed release period (June–July) in 2002. We tagged reproductive plants and conducted between 6 and 13 five-min censuses per plant [540 censuses on 73 plants in total; see Manzaneda *et al.* (2007) for details on sampling protocol]. 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 5-min period. We considered that a seed removal event occurred when ants moved the diaspores beyond the vertical projection of the reproductive stalk on the ground (range 25–90 cm). An instantaneous diaspore removal rate was calculated in each ant

visitor census and for each individual plant as the number of removed diaspores divided by the number of diaspores available per plant in the census. The number of available diaspores in each census was obtained by summing the total number of diaspores on the ground and the number of diaspores in dehiscent carpels on the plant. W_r was then calculated as the instantaneous diaspore removal averaged through censuses for the same plant.

Post-dispersal stages

Post-dispersal fitness components were experimentally estimated for each plant considering two partial scenarios: (1) seeds moved to nests by disperser ants (dispersed seeds) and (2) seeds remaining beneath the maternal plant (undispersed seeds). This approach allows the estimation of sequential post-dispersal fitness components (i.e. predation, germination and survival) separately for dispersed and undispersed seeds. Moreover, it allows the exploration of whether selection on diaspore traits during post-dispersal stages differs for dispersed and undispersed seeds.

To estimate the W_p , we conducted a seed removal trial from seed depots. The experimental units allowed seed removal by mice but prevented removal by ants, ground beetles or birds. Each depot consisted of 20 fresh seeds within a small bag (8 × 8 cm) made of fibreglass mesh that was nailed to the ground. This procedure of exclusion was previously shown to be effective in our study system as mice can easily remove the seeds by chewing the fibreglass threads, whereas ants and other potential removers are unable to do so (Manzaneda *et al.*, 2005a). Thus, after the seed release period (in August–September 2002), we placed beneath each focal plant an experimental depot containing a sample of its own seeds. In addition, in the vicinity of each focal plant with $W_r > 0$ (i.e. plants with some seeds effectively moved), we also placed seed depots in the nests of each species of disperser ant (see Table 1). Seed depots were placed into the nest's waste-midden or close to the nest entrances. Only nests where we visually confirmed that ants brought at least one seed were chosen for the post-dispersal experiments. Seed depots were placed under 73 focal plants, and in 56 nests of seven disperser ant species (Table 1). To minimize the possible interference of alien seeds in the experiment, we manually removed all other seeds that we could find in each microsite (i.e. both beneath the plant and in ant nests). Experimental seed depots were checked after 90 days.

To estimate W_g and W_s , we conducted a seed-sowing experiment with the same setting used to estimate W_p (Table 1). In December 2002, we sowed 20 hellebore seeds beneath our focal plants as well as in the ant nests (only for plants with $W_r > 0$). Each sowing was protected with a 25 × 25 × 8 cm wire-mesh cage to prevent rodent predation. Sowings were checked every month during the emergence period (February–May) and bimonthly

Ant species	Ant size (mu)*	Ant nest midden	No. plants dispersed†	No. nests used in post-dispersal trials‡	Average plant-nest distance (m)§
<i>Campontus cruentatus</i>	62.61	Discrete	38	35	5.60
<i>Aphaenogaster iberica</i>	37.5	Discrete	7	6	3.69
<i>Camponotus vagus</i>	60.0	Discrete	5	5	6.51
<i>Pheidole pallidula</i>	19.6	Diffuse	4	4	0.6
<i>Lasius niger</i>	27	Diffuse	3	3	0.78
<i>Cataglyphis velox</i>	61.9	Discrete	2	2	10.15
<i>Tapinoma nigerrimum</i>	27	Diffuse	1	1	1.2

Table 1 The disperser ant assemblages of *Helleborus foetidus* in the study area.

The number of maternal (i.e. focal) plants whose seeds were dispersed by each ant species is indicated, along with information on ant and nest traits.

*Microscope micrometer units (1 micrometer unit = 0.03125 mm); measurements taken from head width.

†Number of focal plants that were visited by ants and where ants achieved seed removal.

‡Number of nests of each ant species and for each focal plant used in the post-removal experiments.

§Average distance between the focal plant and the ant nests of those ant species involved in the post-removal experiments.

afterwards until October 2004 (end of the first dry period for emerged seedlings). In each survey, we counted and marked the emerged seedlings using coloured bamboo sticks. The survival of the emerged seedlings was monitored until the end of the experiment. To simplify the analyses, we collapsed W_g and W_s into a single component of seedling establishment (W_e).

Total fitness

Total fitness (W_T) can be estimated as the product of plant crop and all k fitness components from seed removal to seedling establishment:

$$W_T = C \prod W_k$$

where C is crop size and $\prod W_k$ denotes the probability for a seed to become an established seedling after surviving its first summer drought (Gómez, 2004). This fitness estimate ignores later stages of the plant reproductive cycle, but includes all relevant stages in which diaspore traits may be directly selected (Alcántara & Rey, 2003). However, as we were interested in exploring the selective advantages of being dispersed, we further considered the contribution to fitness of dispersed and undispersed seeds. Consequently, our estimate of total fitness for each plant considered both contributions using the formulation:

$$W_T = C[(W_r W_{pD} W_{eD}) + ((1 - W_r) W_{pU} W_{eU})];$$

where the subscripts D and U stand for dispersed and undispersed seeds respectively.

Phenotypic selection analyses

Before the analyses, fitness components were normalized to population means to obtain relative fitness components. Diaspore traits were standardized to zero mean

and unit variance. Being relatively small, the sample size of this study ($n = 73$ plants) falls relatively close to the median of the sample sizes in studies of selection reported by Kingsolver *et al.* (2001).

Selection at dispersal

We first analysed phenotypic selection exerted by ants during seed removal on two diaspore traits: seed size and elaiosome size. Because these traits may covary, we examined correlational selection as well as linear and nonlinear selection on these traits by including as independent variables in a multiple regression the multiplicative, simple and quadratic terms of these traits respectively (Lande & Arnold, 1983). In this analysis, the relative seed removal rate was considered the dispersal fitness surrogate.

Post-dispersal selection

Given that no significant selection at dispersal was detected for elaiosome size (see Results), subsequent selection analyses proceeded only for seed size. We estimated standardized selection gradients on seed size for seed removal and post-dispersal stages (escape from predation and seedling establishment), considering the dispersal and nondispersal routes for fitness. Similarly, we estimated the standardized selection gradients for the whole dispersal cycle using the estimates of total fitness. Directional and nonlinear (disruptive and stabilizing) selection gradients were obtained, following the methods of Lande-Arnold (1983), from the regression coefficients of relative fitness on seed size. Directional selection coefficients were obtained by simple regression and the quadratic term was incorporated for estimation of nonlinear selection coefficients. Bootstrap estimates of the regression coefficients and their 95% confidence intervals were obtained in all cases and the coefficient was

considered significant if such interval did not include zero. After identification of a significant negative quadratic term (see Results), indicative of stabilizing selection, we further tested whether the observed population mean lies within the 95% confidence interval for the stationary point on the fitness surface (Mitchell-Olds & Shaw, 1987). To this end, we obtained a bootstrap distribution for the maximum of the fitness surface (maximum = $-b_1/2b_2$, where b_1 is the linear coefficient and b_2 the quadratic coefficient of the fitness function; see Mitchell-Olds & Shaw, 1987). Finally, we additionally considered a cubic-spline to depict the shape of the total selection function. Regressions were conducted with STATISTICA version 7.0 (StatSoft, Inc., 2008), and the bootstrap estimates with the SAS macro JACKBOOT (SAS Institute, 1999). Following Stinchcombe *et al.* (2008), we reported doubled-corrected values of the quadratic selection coefficients.

Exploring different selective scenarios for consequences of seed dispersal

To better comprehend the selective consequences of the seed dispersal mutualism, we simulated two different ecological scenarios of recruitment and their consequences in terms of phenotypic selection. One scenario addresses the consequences of a total failure of seed dispersal in the population (no or collapsed dispersal), so that plant fitness would be a consequence only of post-dispersal fitness components beneath maternal plants. In this case, total fitness for each plant can be estimated as:

$$W_T = C[W_{pU}W_{eU}].$$

The second scenario addresses the consequences of a total dependence of recruitment on seed dispersal, i.e. assuming that all seedlings under the mother plants will eventually die. In this case, total fitness for each plant can be estimated as:

$$W_T = C[W_rW_{pD}W_{eD}].$$

After obtaining the simulated fitness for each plant, phenotypic selection analyses were conducted for each scenario following the procedures described above.

Results

Variation between plants in traits and fitness components

Plants varied notably in crop size (mean \pm 95% confidence limits = 625 ± 136 seeds, ranging between 26 and 3063 seeds; coefficient of variation = 93.5%). Mean seed and elaiosome size were less variable than crop size (CVs of 18.0% and 28.6%, respectively), but their range of variation was still considerable (mean seed size: 11.1 ± 0.5 mg, range 4.4–16.0 mg; mean elaiosome size: 0.7 ± 0.1 mg, range 0.35–1.0 mg). Average seed removal was relatively low ($11.0 \pm 4.4\%$) but highly variable

(CV = 171.9%) and it spanned the full range from zero (23 plants) to 100% (1 plant). The large variation in seed crop and removal resulted in an extremely variable total number of seeds dispersed (92.4 ± 48.2 seeds, range 0–1327 seeds, CV = 223.3%). Crop size was not significantly related to the seed removal rate ($r_s = 0.22$, $P = 0.063$), but it was strongly correlated with the total number of seeds dispersed ($r_s = 0.65$, $P < 0.001$).

Post-dispersal seed predation was generally low, with most seeds escaping seed predation ($87.4 \pm 8.3\%$ for dispersed seeds, and $90.6 \pm 6.1\%$ for undispersed seeds). In contrast, we observed low values of seedling emergence ($24.8 \pm 7.7\%$ vs. $17.8 \pm 6.1\%$, for dispersed and undispersed seeds respectively) and survival ($17.2 \pm 10.2\%$ vs. $16.8 \pm 9.7\%$ for dispersed and undispersed seeds respectively). None of the post-dispersal fitness components differed between dispersed and undispersed seeds (escape from predation: $t_{121} = -0.6$, $P = 0.53$; seedling emergence: $t_{121} = 1.4$, $P = 0.15$; seedling survival: $t_{68} = 0.15$, $P = 0.96$).

Selection at dispersal on seed and elaiosome size

Ants exerted significant selection on seed size, but not on elaiosome size when both traits were considered together (Table 2). No correlational selection was found for these traits. Selection on seed size tended to be disruptive, as denoted by the marginally significant and positive quadratic term (Table 2), but with a marked trend toward plants with large seeds achieving higher removal rates (linear term of seed size significantly greater than zero, Table 2). After removing elaiosome size from the analysis, disruptive selection on seed size during dispersal was confirmed through bootstrap estimates of the selection gradient (see selection for seed removal fitness component W_r in Table 3).

Stage-specific selection on seed size for dispersed and undispersed seeds

For dispersed seeds, there was significant stabilizing selection on seed size during seedling establishment, but there was not significant selection via escape from predation (Table 3). Selection measured through cumulative fitness on dispersed seeds was stabilizing (Table 3),

Table 2 Linear, nonlinear and correlational selection gradients on diaspore traits (seed and elaiosome size) exerted by ant seed removal (seed dispersal to ant nest).

Diaspore trait	Linear ($\beta \pm 1$ SE)	Quadratic ($\beta \pm 1$ SE)	Correlational ($\gamma \pm 1$ SE)
Seed size	0.46 \pm 0.17	0.34 \pm 0.20*	1.02 \pm 0.64
Elaiosome size	-0.22 \pm 0.19	-3.82 \pm 1.94	

Significant selection gradients ($P < 0.05$) are indicated in bold.

* $P = 0.09$.

Table 3 Stage-specific and cumulative selection gradients on seed size through dispersal and nondispersal routes for fitness.

Fitness component	Routes for maternal fitness			
	Dispersed seeds		Undispersed seeds	
	Linear	Quadratic	Linear	Quadratic
Seed removal (W_r)	0.262 (-0.190/0.696)	0.640 (0.032/1.290)	-0.040 (-0.119/0.019)	-0.064 (-0.172/0.010)
Escape to predation (W_p)	0.056 (-0.093/0.212)	-0.125 (-0.343/0.068)	0.021 (-0.036/0.083)	-0.010 (-0.094/0.094)
Seedling establishment (W_e)	0.136 (-0.186/0.453)	-0.581 (-1.156/-0.148)	0.558 (-0.156/1.240)	0.386 (-0.471/1.329)
Cumulative fitness	0.137 (-0.168/0.439)	-0.640 (-1.044/-0.300)	0.718 (0.043/1.229)	0.404 (-0.415/1.330)
	Total fitness (W_T)*			
	Linear		Quadratic	
	0.691 (0.007/1.193)		0.347 (-0.420/1.271)	

The total selection pattern as a result of the sum of fitness obtained through both routes is also shown. Selection gradients with 95% CI (within brackets) of bootstrap estimates not overlapping zero are indicated in bold. Note that in contrast to the stage-specific fitness components, the cumulative and total fitness estimates used in selection analyses incorporate seed crop data (see Methods).

*Sum of dispersed and undispersed seed routes (see text for details).

meaning that plants with intermediate mean seed size were favoured in ant nests. This stabilizing pattern was further asserted by the fact that the observed population mean seed size (11.1 mg) lay within the confidence interval for the maximum of the fitness surface detected through this route (95% confidence interval for the maximum = 10.89–11.60 mg). Thus, a conflict of selection was apparent between ant seed removal and post-dispersal selection, in particular with seedling establishment (Fig. 1). For undispersed seeds, no significant selection was detected in post-dispersal stages, but the selection gradient on seed size for undispersed seeds became directional and positive when considering the cumulative fitness through this route (Table 3). Maternal

plants that produced larger seeds had greater fitness because the probability of seedling establishment increased with seed size. In short, our results illustrate contrasting cumulative selection patterns between dispersed and undispersed routes.

Total selection and mean seed size distribution

Total selection on seed size, estimated as the covariation between plant mean seed size and total fitness (the sum of cumulative plant fitness through dispersed and undispersed seed routes) was significantly directional and positive (Table 3 and Fig. 2). In fact, it resembled the pattern of selection through the undispersed seed route

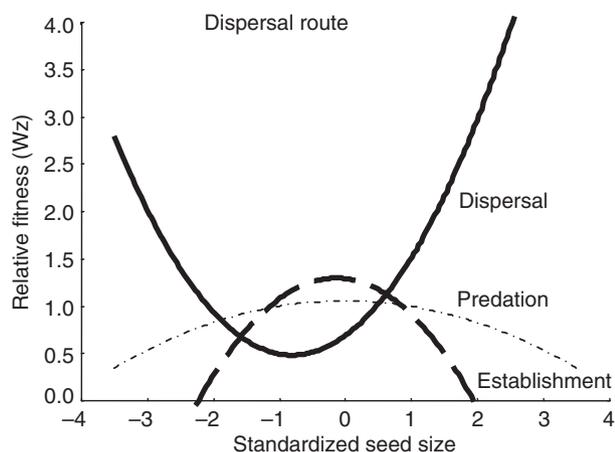


Fig. 1 Fitness functions for selection on seed size at dispersal and post-dispersal stages for dispersed seeds. Significant selection is denoted by thick lines whereas nonsignificant selection is denoted by thin lines (also see Table 3). Fitness components considered are dispersal probability (continuous line), escape from seed predation (dot-dashed line) and seedling establishment (dashed line).

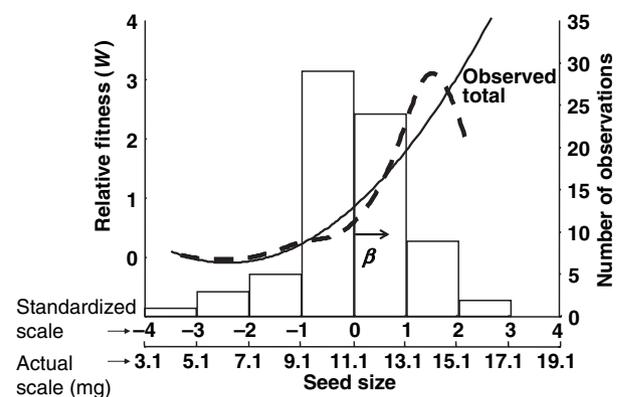


Fig. 2 Total selection on seed size as a result of the sum of the cumulative selection through the dispersed and undispersed seed routes for fitness (continuous line). The result of a cubic spline fitted on total fitness is also shown (dashed line). Selection curves are superimposed on the frequency distribution of plant mean seed size in the population, and the direction of the change in mean seed size provoked by the resultant selection is drawn with an arrow.

(see Fig. 1). This was largely because of the fact that total fitness kept an almost perfect relationship with cumulative fitness through the undispersed seed route ($r_s = 0.99$, $P < 0.001$). In contrast, seed removal rate was unrelated to total fitness ($r_s = 0.124$, $P = 0.3$). Similarly, cumulative fitness through the dispersed route was largely unrelated to total fitness ($r_s = 0.0098$, $P = 0.93$), as well as to the cumulative fitness through the undispersed route ($r_s = 0.039$, $P = 0.75$). Thus, plants with large seeds were favoured as a consequence of the overdominance of the undispersed seed route on fitness.

A cubic spline fitted to total fitness estimates suggests that the modal seed size in the population (Fig. 2) is presently smaller than the optimum predicted by the selection function.

Selective scenarios for consequences of seed dispersal

The two simulated scenarios of consequences of dispersal rendered very different patterns of selection (Fig. 3). The pattern of selection under the scenario of collapsed dispersal was fairly similar to the observed pattern, and the optimum seed size in both cases departs from the observed modal seed size. In contrast, under the scenario of no establishment through the undispersed seed route, the optimum mean seed size matches the actual peak in mean seed size.

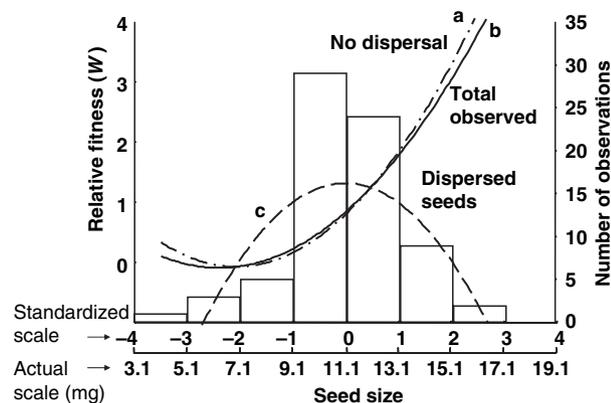


Fig. 3 Total selection on seed size under three different scenarios of consequences of dispersal: (a) no dispersal (dot-dashed line) and fitness only through the undispersed seed route; (b) the seed dispersal observed in the population with seedling establishment through the dispersed and nondispersed route (continuous line) and (c) total recruitment failure through the nondispersed route for fitness (dashed line = dispersed seeds). Selection curves are superimposed on the observed frequency distribution of plant mean seed size in the population. Fitness functions plotted are those obtained from quadratic regression: $w = 0.75 + 0.80s + 0.25s^2$ for no dispersal; $w = 0.84 + 0.78s + 0.16s^2$ for the observed level of seed dispersal; and $w = 1.31 - 0.006s - 0.32s^2$ for dispersed seeds, where s = seed size.

Discussion

Ant seed dispersal is particularly suitable for investigating evolutionary consequences of conflicts of selection on traits that are functional in multiple life stages (such as seed size). Myrmecochory is a directed dispersal system with well-described adaptive advantages (reviewed in Giladi, 2006) where it is possible to know the fate of most dispersed seeds. Here we have shown that seed size but not elaiosome size is subject to selection at the dispersal phase in the hellebore, and that subsequent post-dispersal selective episodes conflict with selection at dispersal, limiting the evolutionary potential of myrmecochory. Moreover, we show that seeds may experience contrasting partial selective scenarios depending on where they are deposited (in ant nests or beneath maternal plants). We show that an analytical procedure combining multiplicative fitness components and microhabitat-specific selection analyses provides a more realistic view of the realized selection and the evolutionary implications of seed dispersal.

Selection at dispersal on size-related diaspore traits in hellebore

Several studies have shown that the size of the elaiosome and the elaiosome to seed ratio may affect the probability of seed dispersal by ants, at least when comparing ant preferences among plant species (Hughes & Westoby, 1992; Mark & Olesen, 1996; Peters *et al.*, 2003). Similarly, other studies have demonstrated a correlation between seed and elaiosome sizes (e.g. Edwards *et al.*, 2006), which is maintained in hellebore across populations (Alcántara *et al.*, 2007). Consequently, correlational selection could be expected between these two diaspore traits. However, we did not detect either simple or correlational selection on elaiosome size, although it was not less variable between plants than seed size. In contrast, we detected selection on seed size that was independent of elaiosome size, indicating that seed size is a key trait determining the probability of dispersal. This result, however, should not be interpreted to mean that seed size is the only important trait to ants, as, for example, elaiosome chemistry has also been shown to affect ant preferences and to be potentially under selection at dispersal in this (Boulay *et al.*, 2006) and other species (e.g. Lanza *et al.*, 1992).

The local ant assemblages exerted disruptive selection on seed size during dispersal, preferentially moving either large or small seeds to their nests. The local ant assemblages in the study area, containing both small and large ants (Table 1), are considerably diverse both taxonomically and functionally (Alcántara *et al.*, 2007; Manzaneda *et al.*, 2007). Typically, small ants prefer diaspores of small size whereas larger ants may disperse a wider range of sizes but still prefer the larger diaspores (Hughes & Westoby, 1992; Mark & Olesen, 1996; Ness *et al.*,

2004). This is also the case in hellebore dispersal (Garrido *et al.*, 2002; Manzaneda *et al.*, 2007). Such patterns of size-dependent preferences may explain the disruptive pattern on selection detected here. Interestingly, this disruptive selection at dispersal seems to be stable in the region as the disruptive pattern has been found in other populations in the study area (Alcántara *et al.*, 2007).

Conflicts of selection on seed size and its evolutionary outcome

Post-dispersal selection on seed size in ant nests follows an opposing pattern to the selection at dispersal, with increased probability of recruitment for medium-sized seeds (i.e. stabilizing selection). This stabilizing pattern may be explained by a detected trend in which plants with larger seeds germinate better in ant nests (bootstrapped directional selection gradient at emergence = 0.34, $P < 0.05$). Consequently, they probably face higher competition in nests than small seeds. In fact, it has been shown in hellebore that seedling mortality is density dependent (Garrido *et al.*, 2005a; Rey *et al.*, 2006).

Thus, an evident conflict of selection is present between dispersal and establishment. The dispersability–survivability conflict is apparently frequent in other dispersal systems like avian seed dispersal of fleshy-fruited plants (Alcántara & Rey, 2003; Martínez *et al.*, 2007), diszoochory (Gómez, 2004; Gómez *et al.*, 2008), and ballistic dispersal (Lázaro & Traveset, 2009). This conflict is related to the more general seed–seedling conflicts (Schupp, 1995), where the best environment for the seed stage rarely is the best for seedlings. The immediate evolutionary consequence of the conflict observed in our study was that the pattern of selection at dispersal was completely reversed at establishment and the final cumulative selection on seed size became stabilizing for dispersed seeds and predominantly shaped at the stage of seedling establishment. Moreover, the strength of cumulative selection on seed size through the dispersal route was -0.64 which is relatively strong as it falls within the lower quartile of the distribution of quadratic selection coefficients reviewed both by Stinchcombe *et al.* (2008) and by Geber & Griffen (2003), the latter specific for functional plant traits, including seed size. To a large extent, the conflict between ant dispersal and seedling establishment limits the evolutionary potential and the ecological consequences of this plant–ant interaction. In fact, some of the presumed advantages of seed dispersal by ants, like the escape from predation and the nutrient enrichment in the nests, were not corroborated here as we found no benefit for dispersed seeds compared to undispersed seeds in these fitness components.

It has been argued that the evolutionary outcome of ant–seed interactions may depend on the species of ant (Giladi, 2006), and consequently the overall pattern of

selection described here could be biased because we did not discriminate among ant species when estimating total fitness. However, we should note that our estimates are largely realistic because they were based neither on a random nor an *ad hoc* selection of ant nests, but instead incorporate the number and identity of the ant species truly dispersing seeds of each plant (Table 1). Thus, while disclosing the contribution of each ant species to the overall selection pattern is an interesting issue, the conflict of selection described here should not be affected by such specificity.

Understanding selection on ecologically important traits during multiple life stages: fitness in different progeny microenvironments

Many studies have demonstrated that seedling recruitment is spatially variable, and there is commonly uncoupling and spatial discordance between stages of recruitment (Herrera *et al.*, 1994; Houle, 1994; Rey & Alcántara, 2000; Garrido *et al.*, 2005a). The implications of such variability for plant population regeneration have been extensively discussed (Houle, 1995; Jordano, 1995; Clark *et al.*, 1999; García *et al.*, 2005; Gómez-Aparicio, 2008), however the evolutionary implications have been largely neglected. That is, microenvironment-specific patterns of phenotypic selection have seldom been investigated in plants, in spite of the general acknowledgement that offspring of each plant reach a range of different microhabitats through dispersal. In fact, although offspring size (i.e. seed mass) and seedling establishment are habitually linked (Gómez, 2004; Rey *et al.*, 2004), information on how microhabitat variability changes the relationship between individual offspring size and performance across life stages is rarely available (but see Tripathi & Khan, 1990; Rey *et al.*, 2004; Gómez *et al.*, 2008). Acknowledgement that phenotypic selection may vary between different microenvironments seems particularly relevant when investigating the evolutionary consequences of dispersal. Here, we have shown that collective consideration of the selective pressures specific to different microenvironments yields a better understanding of the realized selection on seed size in hellebore as well as a more realistic quantification of such selection. Seed size in hellebore was subject to two different partial selective scenarios. Undispersed seeds (i.e. those remaining under the mother plant) were subject to positive directional selection whereas dispersed seeds were subject to stabilizing selection. In both scenarios, the shape of the final selection function was determined by selection occurring during seedling establishment. This did not reflect ant preferences for seed size. Interestingly, the final pattern of selection on seed size was determined not by the dispersed progeny, but rather by the bulk of undispersed seeds.

We advocate the use of this approach, which combines the analysis of multiplicative fitness components and

microenvironment-specific selection, to estimate realistically the realized selection on traits that are functional during several life stages. It may be extended to any organism that disperses progeny to distinct environments.

Ecological and evolutionary roles of seed dispersal mutualisms

The comparison of simulated dispersal scenarios is a useful way to obtain a more realistic picture of the evolutionary implications of dispersal. Total selection under scenarios of observed dispersal rates and collapsed seed dispersal were similarly directional (Fig. 3), as expected from the almost perfect correlation of observed total fitness and cumulative fitness through the undispersed seed route, and move the predicted mean maternal seed size of the population towards larger values. This similarity might be explained by the generally low amount of seed removal achieved in the population (only 11%) and the relatively similar post-dispersal probabilities of seed and seedling survival of undispersed and dispersed seeds. This suggests that dispersal carried no obvious advantage, at least up to early seedling establishment. In fact, there was no correlation between seed removal and total fitness. The scenario of total failure of recruitment through the nondispersing route attempts to simulate the evolutionary result if seedlings from undispersed seeds would not progress in the future because of delayed negative maternal effects on recruitment. That is, these seeds would fail to benefit from the classically expected long-term ecological advantages of dispersal, such as escape from pathogens, seed predators and herbivory, density-dependent mortality near fruiting plants, or colonization of new environments (Howe & Smallwood, 1982; Herrera, 2002). Our simulation shows that an increase in the ecological weight of dispersal would result in a stabilizing pattern of selection, centering the mode, decreasing the variability in seed size, and finally causing a leptokurtic seed size distribution. Interestingly, the observed distribution of mean seed sizes in the population matches this last prediction (the distribution of mean maternal seed sizes is leptokurtic, the mode closely matches that predicted under this scenario and the variance is relatively small), but clearly contrasts with that expected under scenarios of overdominance of the undispersed seed route of fitness or collapsed dispersal. Thus, although many other ecological and evolutionary forces might affect seed size in hellebore (Garrido *et al.*, 2005b; Herrera, 2005 and references therein), the seed size distribution in the population seems in accordance with the stabilizing pattern of selection started by ant dispersal. In short, even though ant selection for seed size is distorted by later-acting selection episodes, the total pattern of selection still depends largely on ant activity, as they control the relative importance of each partial

selective scenario, which ultimately leads to different phenotypic optima.

Acknowledgments

We are indebted to Beni, Cani, Xim, Raphael, Jesús Bastida and José Luis Medina for field work assistance. We also thank Jill Anderson, Prof. Jacqui Shykoff and two anonymous reviewers for valuable comments on the manuscript. Alberto Tinaut identified the ant species. The Junta Rectora of Cazorla, Segura y las Villas Natural Park provided working facilities. Financial support came from Spanish Ministry of Science and Technology Grants BOS2000-1122-C03-02, BOS2000-1122-C03-03, BOS2003-03979-C02-02 and EX-2006-0652.

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Received 22 January 2009; revised 23 March 2009; accepted 24 March 2009