Geographical variation in the potential of mice to constrain an ant-seed dispersal mutualism

J. M. Fedriani, P. J. Rey, J. L. Garrido, J. Guitián, C. M. Herrera, M. Medrano, A. M. Sánchez-Lafuente and X. Cerdá

Fedriani, J. M., Rey, P. J., Garrido, J. L., Guitián, J., Herrera, C. M., Medrano, M., Sánchez-Lafuente, A. M. and Cerdá, X. 2004. Geographical variation in the potential of mice to constrain an ant-seed dispersal mutualism. – Oikos 105: 181–191.

Pre- and post-dispersal *Helleborus foetidus* (Ranunculaceae) seed predation by mice *Apodemus sylvaticus* as well as post-dispersal seed removal by ants was studied, during two years, in six plant populations within three geographical regions (Caurel, Cazorla and Mágina) of the Iberian Peninsula. An observational approach revealed strong interregional differences in seed predation by mice during the pre-dispersal phase, with high and similar rates of predation in Cazorla and Mágina and much lower rates in Caurel. There were also significant inter-annual variations on pre-dispersal seed predation by mice, while the existing habitat-related differences (of lower magnitude) were not consistent across regions. Field experiments based on seed-offering exclosures, showed that, despite some interregional variation, post-dispersal seed removal by ants was consistently high through all spatial and temporal scales considered, with most seeds being removed within 48 h. Conversely, post-dispersal seed predation by mice was highly variable among regions, being very high in Cazorla and minimal or absent in Caurel and Mágina. Interestingly, in Cazorla, in presence of mice, the number of seeds removed was rather independent of the presence/absence of ants, while under mice exclusion, it was determined by the presence/absence of ants. Conversely, in Caurel, the number of seeds removed by each remover agent (ants or mice) was independent of the presence/absence of the other agent. Thus, though uniquely in Cazorla, mice limited the number of seeds available to ants and, therefore, in this region could potentially have interfered on the development of seed traits that enable ants to efficiently harvest them. Our results support the notion that geographical variation over the Iberian Peninsula of mice seed predation may have promoted a mosaic of well-matching and mismatching situations between *H. foetidus* diaspore traits and the characteristics of ant communities, which is consistent with some recent theories on the geographical structure of interactions.

J. M. Fedriani, J. L. Garrido, C. M. Herrera, M. Medrano, A. M. Sánchez-Lafuente, X. Cerdá, Estación Biológica de Doñana, CSIC, Apartado 1056, ES-41080 Sevilla, Spain (fedriani@ebd.csic.es). – P. J. Rey, Depto de Biología Animal, Vegetal y Ecología, Área de Ecología, Universidad de Jaén, ES-23071 Jaén, Spain. – J. Guitián, Depto de Botánica, Facultad de Biología, Universidad de Santiago (USC), Campus Sur, ES-15782 Santiago, Spain.

Seed predation is a central demographic process in plant populations that may influence recruitment, plant species diversity, and plant community structure (Janzen 1971, Crawley 2000, Wenny 2000, Wang and Smith 2002). Intensity and ecological correlates of seed preda-tion are rather species-specific and, even within a given plant species, seed predation patterning may vary enormously in both space and time (Crawley 2000, Hulme and Benkman 2002). Besides, seed predation may occur during both the pre- and/or post-dispersal

181

phases of plant's reproductive cycle (Hulme and Benkman 2002). While pre-dispersal seed predation takes place when seeds are removed from the parent plant before dispersal, post-dispersal seed predation occurs once seeds have been released from the parent plant. Consequently, seed predation may lower the plant seed shadow's overall intensity and also can act as a filter on the resulting seed shadow, modifying seed density and distribution (Hulme and Benkman 2002).

Seed predators may sometimes act as seed dispersal agents, depending on whether they are after the fruit, the seed, or the elaiosome attached to some seeds and depending also on their precision in seed processing (Janzen 1971, Willson and Whelan 1990, Crawley 2000). Both seed predators and seed dispersers are well recognized not only for their potential effects on plant population dynamics but also for affecting the evolution of seed-ripening phenology, morphology, and associated defenses (Janzen 1971, Crawley 2000, Lord et al. 2002). Nonetheless, selection pressures on plants exerted by mutualistic (e.g. pollinators, seed dispersers) and antagonistic (e.g. herbivores, seed predators) interacting organisms often conflict (Herrera 1982, 1984, Benkman 1995, 1999, Brody 1997, Gómez and Zamora 2000); thus, plant adaptations to mutualistic interactions can be compromised by counterselection from antagonistic interactions. Unfortunately, however, comprehensive documentations of this type of interferences are scarce and mostly focussing on pollinator-plant mutualisms (Armbruster 1997, Brody 1997, Gómez and Zamora 2000), while those concerning seed-disperser mutualisms are even more limited (Benkman et al. 2001).

An earlier study carried out in the Iberian Peninsula on the geographic variation in the interaction of *Helleborus foetidus* (Ranunculaceae) seeds with its ant dispersers found a mosaic of well-matching and mismatching situations between diaspore traits and the structural and functional characteristics of local ant communities (Garrido et al. 2002). This finding is consistent with some recent theories on the geographic structure of interactions (Thompson and Pellmyr 1992, Thompson 1994, 1999) postulating that interspecific interactions vary in outcome from one selective scenario to another. Furthermore, variation in such selective scenarios is often determined by geographical variation in the strength of the effect of natural enemies associated with interacting species (Benkman 1995, 1999, Gómez and Zamora 2000, Herrera et al. 2002, Leimu et al. 2002, Stinchcombe and Rausher 2002). Therefore, assessing the geographical pattern of seed predation experienced by populations of *H. foetidus* could help to understand the selective scenarios in which some seed-disperser mutualisms are embedded and, thus, to identify the processes underlying the patterning of geographical variation in the outcome of those mutualisms.

In this study, by considering six *H. foetidus* populations widely distributed over the Iberian Peninsula, we evaluate whether interpopulation shifts in intensity of both pre- and early post-dispersal seed predation by mice could lead to differing levels of seed availability for ants and thus to interfere in a variable manner across populations in the ant–seed mutualism. This study is aimed to address three specific objectives: 1) to evaluate the pervasiveness of the interaction ant–*H. foetidus* and to quantify regional, habitat-related, and annual variations in the strength of the mutualism, 2) to evaluate the importance of rodents as pre- and post-dispersal seed predators of *H. foetidus* and to quantify the regional, habitat-related, and annual variation in the strength of such antagonistic interaction, and 3) to evaluate whether post-dispersal seed predation by mice could interfere in the ant–seed mutualism by limiting the number of seeds available to ants and, if this interference occurs, to assess its spatial and temporal consistency.

Study system and sites

Helleborus foetidus L. (Ranunculaceae) is a perennial herb widely distributed in western Europe (Werner and Ebel 1994). In the Iberian Peninsula, the species typically grows in clearings, understory of deciduous, mixed forest, and in scrubland. Plants consist of several (1–5) ramets that develop terminal inflorescences after several years. In winter (January–March), each inflorescence usually produces 25–75 flowers that are predominantly bumblebee-pollinated (Herrera et al. 2001, 2002a). Fruits may have up to 5 carpels (usually 2–3) and fruit maturation and seed shedding take place in June–July, when each carpel release 8–15 elaiosome-bearing seeds. Diaspore mass ranges 5–19 mg (Garrido et al. 2002). The species does not show marked physical defense of their flowers and fruits against predation by mice, which is most likely related to its reliance on chemical defense (Werner and Ebel 1994).

Our study was carried out during 1998 and 1999 at six populations within three geographical regions (Caurel, Cazorla, and Mágina, Herrera et al. 2001), encompassing a considerable part of the range of *H. foetidus* in the Iberian Peninsula and coinciding with three out of the five regions studied by Garrido et al. (2002). The two more distant regions (Cazorla-Caurel) were ~675 km apart, while the two nearest ones (Cazorla-Mágina) were ~85 km apart. Because seed removal is often habitat-dependent (Willson and Whelan 1990, Hulme and Benkman 2002) and to encompass a representative range of ecological conditions (vegetation cover, alternative food, seed remover abundances) in which *H. foetidus* occurs, for each region we chose two populations of *H. foetidus* located in different habitats (called "woodland" and "open" populations, respectively). In Caurel,

the "open population" occupies an open successional scrubland and *Brachypodium rupestre* meadows, and the "woodland population" is located within a dense mixed forest of *Castanea sativa*, *Betula alba* and *Acer pseudoplatanus*. Both populations grew at 1150 and 950 m elevation. In Cazorla, the "open population" occupies a sparsely vegetated area with scattered oak (*Quercus rotundifolia*) and pines (*Pinus nigra*). The "woodland population" is located within a mixed forest of pine (*Pinus nigra*) and oaks (*Quercus rotundifolia*) with understory of sparse *Juniperus oxycedrus*. In Mágina, the "open population" is located within an open area with low developed shrub layer of *Crataegus monogyna*, *Rosa canina* and *Berberis vulgaris* with grass understory and some scattered *Quercus coccifera*. The "woodland population" is located within dense mixed overstory of Q. *rotundifolia* and *Quercus faginea* with medium developed understory of *Rosa canina* and *Crataegus monogyna* bushes. Populations of these two southern regions (Cazorla and Mágina) grew at 900–1600 m of elevation. Percentage of canopy cover was estimated for each population as the difference between the number of pixels of hemispheric photographs (n = 30–34 pictures per population) directly exposed to solar radiation minus the total number of pixels/total number of pixels × 100. Mean values ($\pm$1SD) of these estimates were 34.5%$\pm$16.7 and 42.0%$\pm$8.5 (Caurel), 58.5%$\pm$10.1 and 61.7%$\pm$9.6 (Cazorla) and 11.2%$\pm$11.3 and 40.4%$\pm$18.9 (Mágina) for open and woodland populations, respectively. Though the absolute magnitude of difference in canopy cover between the open and woodland populations within regions was not consistent across regions, open populations were always of lower canopy cover as compared with the woodland populations.

Garrido et al. (2002) documented the composition of the ant communities, abundance of individual species, and their functional role (seed dispersers vs granivores) in the same three regions considered in this study. Most of the 33 species recorded in that study were efficient seed dispersers, and granivorous ants were rare in all regions. The most common species belonged to the genera *Aphaenogaster*, *Crematogaster*, *Formica*, *Lasius* and *Myrmica*. Local rarity of granivorous ants was confirmed during the summer of 1998 by systematic pit-fall trapping in all six *H. foetidus* populations (authors, unpubl.). Small mammal trapping carried out during a parallel project has shown both that field mouse *Apodemus sylvaticus* is the predominant rodent species in all three regions and also that mice abundance at Cazorla is considerably higher than in the other two regions; however, we caution that such trapping efforts were carried out during the years succeeding this study (2000–2003; authors, unpubl.) and that mice populations may show pronounced interannual variations.

Methods

A previous study has found that mice may feed on both developing fruits and open flowers of *H. foetidus* (Herrera et al. 2002b). While developing fruits provide mice with seeds, flowers provide in their nectaries abundant nectar available to mice (Herrera et al. 2002b, Rey et al. unpubl.). Mice generally feed on both plant structures (fruits and flowers) by following a similar foraging procedure, i.e. by chewing the pedicels and removing the whole structure from the parent plant; therefore, sometimes can be problematic to assess whether a given structure was removed from the plant during the flowering or fruiting period. For this reason and for the sake of simplicity, in the following analyses, we will treat and present the results of "pre-dispersal fruit and flower losses" jointly. On the other hand, after seed shading, mice remove *H. foetidus* seeds scattered in the surroundings of parent plants, which will be referred hereafter as "post-dispersal seed removal".

Pre-dispersal fruit and flower losses

Pre-dispersal fruit and flower losses by mice was evaluated in each region by considering 18–35 plants per habitat during both 1998 and 1999. Plants (overall, n = 358) were randomly chosen at the starting of flowering season (January) and monitored (3–5 times per plant) to the end of the ripening season (June–July). For each plant, we recorded its final fruit production and fruit plus flower losses due to mice. Fruit and flower losses due to mice refer to the proportion of fruit plus flowers removed by mice with respect to total available at plants. Number of available fruits plus flowers was estimated for each plant as the number of fruits releasing seeds at the end of the fruiting period plus the cumulative number of fruits and flowers removed by mice. Since the number of fruits and flowers available at monitored plants was very variable (see below) and it could affect mice predation (Sallabanks 1993), we accounted for its possible effect on seed predation by including it in our model as a covariable.

Post-dispersal seed removal

To evaluate the importance of ants and mice as post-dispersal seed removers and to quantify their regional, habitat-related, and annual variations, we quantified separately rates of diaspore (seed + elaiosome; but hereafter referred to as "seeds") removal under a 2 × 2 experimental design in which both "Ants" and "Mice" were the main factors, each one with two levels (presence/exclusion). Thus, seeds were exposed to four treatment combinations and each set of four treatment combinations formed each "experimental unit". The

treatments were: 1) ant+mice: seeds were presented on an upturned petri dish, fixed with a nail flush with ground surface and were available to both mice and ants (each dish presented in their rim two holes at the floor level to facilitate ant access), 2) ants alone: seeds were presented in the same type of petri dish but totally covered by a 1 cm wire mesh and, therefore, were available to ants only (Hulme 1997). No evidence of seed losses due to any other potential seed remover than ants nor to other factors (wind, rainfall) was found in these exclosures, 3) mice alone: to enable seed access to mice but not to ants, the rim of a petri dish was painted with a Teflon emulsion (Fluon, Hulme 1997). Though birds were not excluded by this means, our two-checking-per-day schedule followed during these seed offerings (and also on posterior similar field experiments) indicated clearly that diurnal birds did not remove *H. foetidus* seeds, and 4) full exclusion: to prevent the access of both mice and ants, the same type of petri dish was totally covered by a 1-cm wire mesh and the rim of a petri dish was painted with a Teflon emulsion (Fluon). Each experimental unit was replicated 15 times per region and habitat, during both 1998 and 1999, and were set separated by 2–3 m within the same *H. foetidus* populations chosen for the pre-dispersal predation assessment and in a way that maximized the spatial overlap with the distribution of plants monitored previously. Within each experimental units, the exclosure treatments were placed apart 40 cm of each other in randomly designated positions. Dishes were always set out of bushes and in sites with little or none understory in order to set them under similar "microhabitat" and thus control for its potential effect on different seed removers (Hulme 2002, Rey et al. 2002). In each dish, ten fresh seeds of *H. foetidus* were placed and left in place for up to four days (96 h). Dishes were checked approximately every twelve hours (i.e. immediately before dusk and after dawn) and the number of seeds removed was recorded. To make results comparable across regions, the variable response used was always the number of seeds removed by either mice or ants within the first 48 h. The experiments were undertaken during the natural seed dispersal period of *H. foetidus* at each locality (June–July).

Statistical analyses

We evaluated the potential effect of geographical region, habitat type and year on the number of available fruits and on pre-dispersal fruit and flower losses by fitting generalized lineal models using SAS macro GLIMMIX (Littell et al. 1996, Herrera 2000). This program iteratively calls SAS procedure Mixed that allows the modeling of the response variable as Poisson (e.g. number of fruits plus flower available) or binomial (e.g.

percentage of fruits plus flowers predated) processes. Region, habitat and year and all possible two-way interactions among these three main factors were considered as fixed effect in the model. Habitat was considered a fixed effect (and not a random effect nested within region) because both levels of this factors (i.e. "open" and "woodland") were the same in all three regions. Further, these two habitats were not chosen at random (Bennington and Thayne 1994) and seed predation was a priori expected to be habitat-depend (Hulme and Benkman 2002). When the interaction between any two variables was significant, GLIMMIX macro allows to perform tests for the effect of a given factor tested at the different levels of the other factor ("tests of simple main effects", Schabenberger et al. 2000) using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell et al. 1996). This SLICE option is not available in other SAS procedures allowing the modeling of non-normal response variables (GENMOD). LSMEANS statement provided model-adjusted means and standard errors in the scale determined by the chosen link-function. Standard errors of such means were back-transformed using the appropriate Taylor's series approach (Littell et al. 1996).

Information derived from the post-dispersal seed offerings was also analyzed by fitting generalized lineal model using SAS macro GLIMMIX (Littell et al. 1996). Since the response variable was the cumulative number of seeds removed over 48 h, we fitted the model with Poisson error and log-link function. As mentioned above, we used a 2×2 experimental design in which the two main factors were ants (presence/exclusion) and mice (presence/exclusion). Region, habitat and year and all possible two-way interactions among these three factors were also considered as fixed effect in the model. In addition, to evaluate whether mice limited the number of seeds available to ants and to assess the spatial and temporal consistency of such potential interference ("objective 3" in Introduction), we also included in the model the interaction mice × ants, as well as the three-way interactions mice × ants × region, mice × ants × habitat and mice × ants × year. As in the previous analysis, when the interaction between any two variables was significant, we performed "tests of simple main effects" by using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell et al. 1996).

Results

Pre-dispersal fruit and flower losses

We monitored fruit production and losses on 121, 110, and 127 plants in Caurel, Cazorla and Mágina, respectively, which overall produced 12 256 fruits plus flowers available to mice. There were strong inter-regional

differences in fruits plus flowers availability of monitored plants (Table 1), averaging 12.4±1.4 fruits plus flowers (mean per plant ±1 SE), 28.5±2.0, and 49.2±2.5 for Cazorla, Caurel, and Mágina, respectively. Further, mean fruits plus flowers availability during 1998 (19.3±1.6) was significantly smaller than during 1999 (34.8±1.7, Table 1). These estimates of the magnitude of the differences in fruits plus flowers availability among regions and between years would be meaningful only if these two factors (i.e. region and year) did not have a joint effect (interaction) on the number of fruits plus flowers available. This was not the case in the present instance. The interaction region × year was statistically significant (Table 1), indicating that the sign and/or magnitude of the effect of either of these main effects depended on the level of the other factor. Thus, while in Caurel and Cazorla the number of fruits plus flowers available was clearly higher during 1999 (Fig. 1A), in Mágina there were not such differences. Indeed, tests of simple main effects (or interaction slices) indicated that differences between years were statistically significant in Caurel and Cazorla but not in Mágina (Table 2). Finally, we did not find habitat-related differences in the number of fruits plus flowers available (Table 1).

Of the overall fruits plus flowers available in all six populations (n = 12 256) mice preyed on 3733 (255, 798, and 2680 in Caurel, Cazorla and Mágina, respectively). As expected, number of fruits plus flowers available had an effect on the proportion of fruits predated (Table 1). Specifically, the positive sign of the "parameter estimate" for fruits plus flowers availability (0.006±0.002, mean±1 SE) indicated that plants with larger crops were proportionally more predated. After statistically accounting for the effect of fruits plus flowers available, all three main effects significantly influenced pre-dispersal fruit and flower losses by mice (Table 1), with year and region being the major sources of variation. Corrected mean predation rates for Cazorla and Mágina were relatively similar and much larger than those for Caurel (45.6%±9.4 and 34.7%±5.8 vs 4.0%±6.6, respectively). Overall, "open populations" suffered higher predation than "woodland populations"; how-
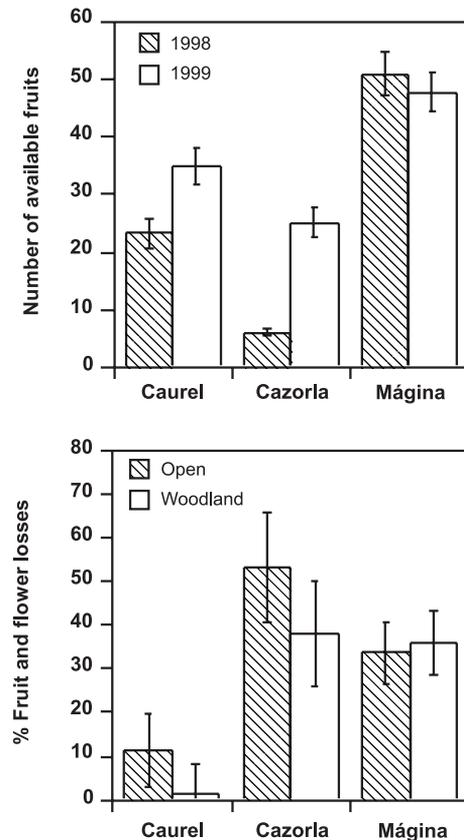

Fig. 1. (A) Corrected mean number of available fruits (±1 SE) in monitored *Helleborus foetidus* L. (Ranunculaceae) plants in three geographical regions (Caurel, Cazorla, and Mágina) within the Iberian Peninsula during 1998 and 1999. In each region two *H. foetidus* populations were chosen, in open and woodland habitats respectively. (B) Corrected means (±1 SE) of percentages of pre-dispersal fruits and flower losses by mice.

ever, the interaction region × habitat was statistically significant (Table 1), indicating that sign and/or magnitude of the effect of either of these main effects depended on the level of the other factor. Thus, while in Caurel and Cazorla pre-dispersal fruits plus flowers losses by mice was much higher in the "open" habitats than in the

Table 1. Significance of the effects of region, habitat and year and their two-way interactions on both the availability of fruits and on fruit predation using generalized linear models. The model for fruit availability was fitted using Poisson error and a log link function. The model for pre-dispersal seed predation was fitted using binomial error and a logit link function. In this second model the availability of fruits was included as a covariate. All significant results in boldface (p < 0.05).

	Fruit availability			Fruit predation		
	df	F	P	df	F	P
Region	2, 383	62.09	**< 0.0001**	2, 347	31.53	**< 0.0001**
Habitat	1, 383	2.17	0.141	1, 347	15.13	**0.0001**
Year	1, 383	28.57	0.0001	1, 347	47.81	**< 0.0001**
Region × habitat	2, 383	0.17	0.846	2, 347	7.81	**0.0005**
Region × year	2, 383	9.78	**< 0.0001**	2, 347	1.13	0.324
Habitat × year	1, 383	2.12	0.146	1, 347	1.10	0.295
Number of fruits	–	–	–	1, 347	11.66	**0.0007**

Table 2. Summary of tests of simple main effects (or "interaction slices"; SAS Institute 1996) for (a) the effect of year on the number of available fruits within each geographical region (i.e. interaction year × region in Table 1), and for (b) the effect of habitat on the percentage of pre-dispersal fruit and flower losses by mice within each geographical region (i.e. interaction habitat × region in Table 1). All significant results in boldface (p < 0.05).

	df	F	P
a) Fruit availability			
Year in Caurel	1, 383	8.43	**0.004**
Year in Cazorla	1, 383	39.64	**< 0.0001**
Year in Mágina	1, 383	0.37	0.542
b) Pre-dispersal fruit and flower losses			
Habitat in Caurel	1, 347	12.95	**0.0004**
Habitat in Cazorla	1, 347	3.60	0.059
Habitat in Mágina	1, 347	0.29	0.592

"woodland" habitats, in Mágina there were not differences between habitats (Fig. 1B). Tests of simple main effects indicated that differences between habitats were highly significant in Caurel, marginally significant in Cazorla and not significant in Mágina (Table 2). Finally, mean rates of pre-dispersal fruit and flower losses during 1998 (29.6% ± 5.9) doubled those found for 1999 (14.2% ± 7.6), being this difference statistically significant (Table 1).

Post-dispersal seed removal

For descriptive purposes and to present evidence of the relative effectiveness of the exclosures used in the post-dispersal seed offerings, we provide a graphic representation of the number of seeds remaining after every 12 h ("seed survival curves", Fig. 2). Thus, for example, the curves corresponding to "Ants treatment" show that ants were consistently the major seed removers, removing most seeds within 48 h in most regions, habitats and years (Fig. 2). The "full exclusion curves" combined with our observations of the seed depots during the seed offerings revealed that the exclusion of mice was always effective while the exclusion of ants failed in some experimental units, possibly due to particular in situ conditions (e.g. unusual high local ant abundance). Ant exclusion failure was rare in Cazorla and Caurel (23 out of 120 experimental units failed), whereas in Mágina was rather general; therefore, data from those experimental units from Cazorla and Caurel for which we recorded ant exclusion failure were excluded from analyses. Further, due to general failure in ant exclusion at Mágina, analyses of post-dispersal seed removal were initially performed without considering data from this region (see below). In addition, survival curves for "mice treatment" in Mágina were never below the curves for seeds under "full exclusion" (Fig. 2), indicating that seed removal by mice in Mágina was absent or at least very
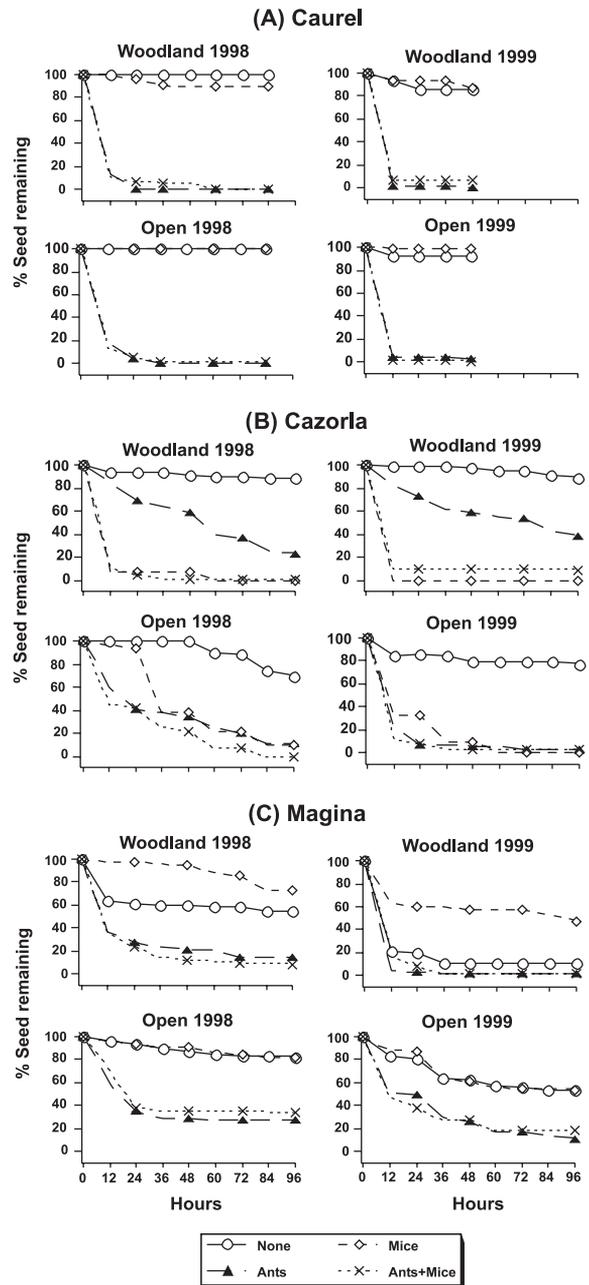

Fig. 2. Number of *H. foetidus* seeds exposed to ants and rodents remaining in petri dishes during up to 96 h in two habitats (open and woodland, respectively) within three regions (A, Caurel; B, Cazorla; C, Mágina) and two years (1998 and 1999). Each treatment indicates to which remover agent seeds were exposed (control = full exclusion). Because failure of ant exclusion was very pronounced in Mágina (C), data for this region was not considered in some analyses (see Methods for details).

low in both habitats and both years. In Cazorla the curves corresponding to "mice treatment" reveal that mice were important seed removers, while in Caurel seed removal by mice was minimal.

Because of widespread failure of ant exclusions in Mágina, analyses of potential differences in the "number of seeds removed within 48h" (hereafter referred to as "NSR") were first done considering exclusively Caurel and Cazorla. Using this "partial" data-set, we found inter-regional differences in the NSR by ants (interaction ants × region, Table 3). Even though the NSR by ants was high in both regions, it was greater in Caurel than in Cazorla (Fig. 3A). As would be expected from the high NSR by ants in both regions, test of simple main effects indicated that ants had a significant effect on the NSR in both regions (Table 4). In addition, seed survival curves suggest that seed removal by ants was faster in Caurel as compared with Cazorla, especially in the "woodland habitat" (Fig. 2). The NSR by mice also differed strongly between regions (interaction mice × region, Table 3), being much smaller in Caurel than in Cazorla (Fig. 3B). Tests of simple main effects indicated that mice did not have an effect on NSR in Caurel while in Cazorla they had a strong effect (Table 3, Fig. 3B). We also found a highly significant interaction ants × mice (Table 3), indicating that NSR by each remover agent (ants or mice) did depend of the presence/absence of the other agent. More importantly, the significance of the ants × mice × region three-way interaction indicated that the interaction ants × mice was not homogeneous across regions. Separate graphs for the two regions (Fig. 4) show clearly the inconsistency of the ants × mice interaction. In Caurel (Fig. 4), NSR was independent of the presence/absence of mice and clearly dependent only on the presence/absence of ants, suggesting the lack of potential of mice to limit the number of seeds available to ants and thus to interfere in the ant–seed mutualism.
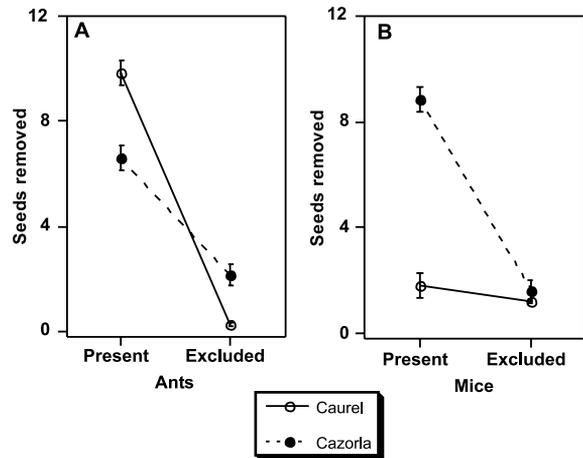

Fig. 3. Graphical representation of the interaction ants × region and mice × region showing the corrected mean number (± 1 SE) of seeds removed after 48 h by ants and mice in presence and absence of the other remover agent in Caurel and Cazorla.

Conversely, in Cazorla (Fig. 4), in presence of mice, NSR was rather independent of the presence/absence of ants, while under mice exclusion ants determined the NSR. These results indicate that under natural conditions (i.e. access of both mice and ants) mice in Cazorla limited the number of seeds available to ants and, therefore, mice showed potential to interfere on the ant–seed mutualism.

Finally, when data from Mágina are included in the analyses, regional differences in the effect of both ants and mice persisted (Table 3). Tests of simple main effects

Table 3. Analyses of post-dispersal *H. foetidus* seed removal by mice and ants by using generalized linear models. The variable response (number of seeds removed within 48 h) was assumed to follow a Poisson error and thus a log link function was used in the models. Because failure in ant exclusion in Mágina the analysis was first performed without considering this region and thus the interactions mice × ant, mice × ants × region, mice × ants × habitat and mice × ants × year could be accurately tested. In a second analysis, we considered also data from Mágina and found a consistency of the inter-regional differences in seed removal detected in the previous analysis. All significant results in boldface (p < 0.05).

	Caurel and Cazorla			All three regions		
	F	df	P	F	df	P
Ants	157.85	1, 394	**< 0.0001**	233.83	1, 633	**< 0.0001**
Mice	28.50	1, 394	**< 0.0001**	58.55	1, 633	**< 0.0001**
Region	25.42	1, 394	**< 0.0001**	45.4	2, 633	**< 0.0001**
Habitat	0.39	1, 394	0.533	23.91	1, 633	**< 0.0001**
Year	2.84	1, 394	0.093	15.36	1, 633	**< 0.0001**
Region × year	0.27	1, 394	0.601	0.42	2, 633	0.654
Habitat × year	1.14	1, 394	0.287	0.17	1, 633	0.681
Ants × region	51.98	1, 394	**< 0.0001**	58.19	2, 633	**< 0.0001**
Ants × habitat	1.11	1, 394	0.293	23.11	1, 633	**< 0.0001**
Ants × year	1.36	1, 394	0.244	9.33	1, 633	**0.002**
Mice × region	13.19	1, 394	**0.0003**	38.60	2, 633	**< 0.0001**
Mice × habitat	0.04	1, 394	0.833	0.26	1, 633	0.612
Mice × year	1.01	1, 394	0.316	0.09	1, 633	0.769
Mice × ants	14.79	1, 394	**< 0.0001**	–[a]		
Mice × ants × region	4.10	1, 394	**0.043**	–[a]		
Mice × ants × habitat	0.53	1, 394	0.467	–[a]		
Mice × ants × year	0.12	1, 394	0.731	–[a]		

[a] No tested due to failure in ant exclusion in Mágina.

Table 4. Tests of simple main effects (or "interaction slices"; SAS Institute 1996) for the separate effect of ants and mice on the number of seed removed during the post-dispersal phase within each geographical region (i.e. interactions ants × region and mice × region). Because generalized linear models were initially fitted without considering data from Mágina (see Methods), we first performed tests of simple main effects considering only data from Caurel and Cazorla and then considering all three regions. All significant results in boldface (p < 0.05).

	Caurel and Cazorla			All three regions		
	df	F	P	df	F	P
Ants in Caurel	1, 394	137.27	**< 0.0001**	1, 632	169.23	**< 0.0001**
Ants in Cazorla	1, 394	31.82	**< 0.0001**	1, 632	21.73	**< 0.0001**
Ants in Mágina	–	–	–	1, 632	67.34	**< 0.0001**
Mice in Caurel	1, 394	1.34	0.248	1, 632	0.01	0.926
Mice in Cazorla	1, 394	73.28	**< 0.0001**	1, 632	103.76	**< 0.0001**
Mice in Mágina	–	–	–	1, 632	1.06	0.304

revealed that ants had an effect in all three regions while rodents only had a significant effect in Cazorla (Table 4), which corroborates patterns reported above. In addition, this analysis evidenced some habitat-related and interannual differences in seed removal by ants which were not found in the previous analysis; however, because of the "noise" caused by the failure in ant exclusion in Mágina such results must be taken with caution and, therefore, are not presented here in further detail.

Discussion

The ant–seed mutualism

This study evidences the pervasiveness of the mutualism ant–*H. foetidus* and reveals a high strength in the interaction (i.e. high seed removal rates) that, despite some variation in its magnitude, keeps consistently high through all spatial and temporal scales considered. Rates

Fig. 4. Separate graphs for the interaction mice × ants in Caurel and Cazorla. In Caurel, the overall number of seeds removed did not depend on the presence/absence of mice while in Cazorla this number was much smaller when mice were excluded. These results show both the potential of mice to limit the number of seeds available to ants in Cazorla and the lack of such potential in Caurel.

of ant seed removal of the sort reported here are frequent for similar sized seeds (Brown and Davidson 1976, Brown and Heske 1990). Local communities of ants are mostly composed by species that feed on the elaiosome attached to seeds rather than by granivorous species (Garrido et al. 2002, authors, unpubl.); thus, as commonly reported in temperate ecosystems (Hulme and Benkman 2002), most seeds removed by ants were subsequently dispersed but not predated. Importantly, our design of seed depot offerings incorporated both components of seed predation; say, seed encounter and seed exploitation (Hulme and Hunt 1999). Certainly, foraging ants had first to find the seed depots and, once found, to exploit them. Indeed, our data showed that ants behaved as very efficient foragers of *H. foetidus* seeds, with most seed depots being found and totally exploited within 48 h. Therefore, this study rather than merely corroborate results from Garrido et al. (2002), which did not incorporate seed encounter in their approach (as seeds were directly presented to foraging ants), reflects most comprehensively the pervasiveness and strength of myrmecochory in *H. foetidus* and is consistent with the notion that ant assemblages from different regions and habitats may be ecologically equivalent (sensu Zamora 2000). On the other hand, our seed offering protocol, which was held constant in all regions and years, reflects rather accurately the field conditions in which ants (and mice) ordinarily found seeds and, in agreement with previous studies employing similar exclosures (Hulme 1997, Kollmann et al. 1998, Hulme and Hunt 1999), we suggest that exclosures had negligible effects on seed movements. Thus, we would expect that under field conditions, at least in populations where mice are absent or scarce (see below), a large fraction of seeds released from *H. foetidus* plants would be regularly found and dispersed by ants. Consequently, it is unlikely that the overall lack of adjustment between seed traits and structural and functional ant community parameters found by Garrido et al. (2002) was related to a low strength, inconsistency or unpredictability in the interaction, and it seems reasonable to expect that factors extrinsic to the ant–seed interaction promoted such findings.
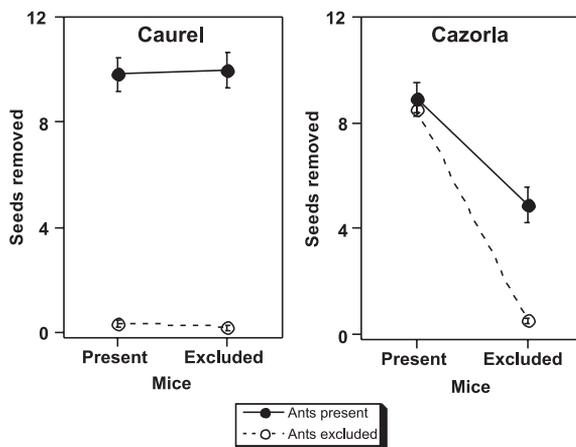
188

Variation in seed removal by mice

This study shows pronounced spatial and temporal variations in intensity of pre-dispersal fruit and flower losses caused by mice, corroborating that such strong variation is the norm in nature (Crawley 2000). Spatial differences were of greater magnitude between regions than between habitats in the same region, strengthening the importance of large-scaled studies in assessing the role of mice seed predation. Further, habitat-related differences in pre-dispersal fruit and flower losses were not consistent through the three regions (Fig. 1B), suggesting that factors non-accounted for in the pre-dispersal assessment (e.g. microhabitat) could have a major influence in mice foraging rather than macro-habitat. Our results also show strong interannual differences in pre-dispersal predation by mice despite the short time frame considered (two years). Greater differences on pre-dispersal fruit and flower losses caused by mice would be most likely found on a longer time span (Wang and Smith 2002). As it has been found elsewhere (Hoshizaki and Hulme 2002), these interannual differences on mice predation could be related to annual variations in availability of alternative foods. Indeed, our own field observations and data gathered by colleges suggest that low incidence of mice *H. foetidus* seed predation during 1999 could be linked to a high availability of acorns and pine cones during the preceding winter (authors, pers. obs., F. Pulido, pers. comm.).

As during the pre-dispersal phase, post-dispersal seed removal by mice was also very variable in space, being absent or minimal in Caurel and Mágina but very high in Cazorla (Fig. 2). High spatial variability in mice post-dispersal seed predation is also common (Kollmann et al. 1998, Edwards and Crawley 1999, Alcántara et al. 2000, Hulme 2002, Rey et al. 2002). However, low post-dispersal seed removal rates by mice in Caurel and Mágina contrast with those found for other plant species elsewhere (Hulme and Borelli 1999, Alcántara et al. 2000). On the other hand, recent reviews report that post-dispersal seed predation is, on average, more severe than pre-dispersal predation (Hulme 2002, Hulme and Benkman 2002); this study, however, reveals lower rates and restricted range of post-dispersal removal as compared with pre-dispersal removal. Such result is likely related to the lower reward and inconspicuousness of individual seeds as compared with the high reward of aggregated fruits located on conspicuous and relatively accessible parent plants (Crawley 2000). Nonetheless, we caution that the "open microhabitat" in which seed depots were set may have lowered mice seed removal as rodents often prefer to forage in densely vegetated microhabitats (Fedriani et al. 2002, Hulme and Benkman 2002).

A variety of factors such as mice density, their food preferences, and availability of alternative foods can be the proximate causes underlying the strong spatial and temporal differences in seed predation reported here. Interestingly, some of these factors (mice abundance, alternative foods) are likely related to changing weather conditions. For instance, periodic weather episodes, such as "El Niño" southern oscillation (with known repercussions in Europe too, Brázdil and Bíl 2000), may influence rodent populations (Jaksic et al. 1997) and seed recruitment by trees (Connell and Green 2000). Therefore, a myriad of "external" drivers could be exerting a critical role in the ant–seed mutualism, which certainly deserves further research. In this study, rather than to identify the relative importance of those factors, we have focused on whether high strength of mice seed predation found in some of our target populations could locally limit the availability of seeds to ants and thus lessen the strength of myrmecochory. That issue and whether or not the strong variations in mice seed predation could promote contrasting selective scenarios for the ant–seed mutualism are discussed below.

The role of mice in the dynamics of *H. foetidus* and in the ant–seed mutualism

Despite intensive searches in some of the populations studied, there was no evidence that mice cached fruits of *H. foetidus* for later consumption (Smith and Reichman 1984, Vander Wall 1990, 2002, 2003). Further, surviving seeds were not found either beneath predated plants or within rock crevices where, however, presence of carpel remains and seed coats (testa) were frequently found. Our observations are consistent with the small size of *H. foetidus* seeds, given that the benefit derived from caching augment with the size of the stored food (Vander Wall 1990, 2003). Therefore, it is likely that mice acted exclusively as seed predators (no dispersers) and, in populations with high rates of pre-dispersal fruit and flower losses by mice (Cazorla and Mágina), they significantly decreased the total number of seeds released and lowered the plants seed shadow's overall intensity, limiting thus plant's recruitment (Herrera et al. 2002b). Importantly, by lessening the fitness of plants and limiting the number of seeds that subsequently would be dispersed by ants, mice likely reduced the chance for selection by this mutualistic agents. Besides this, individual plants varied enormously in their rates of pre-dispersal fruit and flower losses by mice, that ranged 0–100% (Rey et al. unpubl.). If this variation were based on heritable traits, predation pressure exerted by mice on populations of *H. foetidus* in Cazorla would affect the relative representation of different genotypes in future generations, having thus an additional impact on the selective processes among ants and *H. foetidus* seeds. Further studies are needed to evaluate this possibility.

In Cazorla, during the postdispersal phase, mice also constrained the interaction ant–seed by acting as a prior

"filter" on the resulting seed shadows, lowering seed densities and distributions, and making sites distant away from the parent plants that received less seeds (Hulme and Benkman 2002). Besides, the potential of mice to constrain seed dispersal by ants is reinforced by the facts that in Cazorla mice limited the number of seeds removed by ants and also that in such *H. foetidus* populations ants showed the slowest rates of seed removal (Fig. 2) providing mice with longer time spans to harvest seeds and thus to outcompete ants. Finally, insofar as post-dispersal seed removal by mice is related to heritable seed traits (e.g. diaspore mass), mice could affect the relative representation of different genotypes in future generations and those genotypes could contrast with genotypes selected by ants, enhancing further the potential of mice to interfere in the ant–seed mutualism.

Variation in fruit abortions due to abiotic factors as well as fruit losses due to invertebrates (lepidopteran larvae) and fungi could also limit the reproductive ability of *H. foetidus* and thus interfere on its mutualistic interaction with ants. However, mice seem the only abundant and ubiquitous agent capable of constraining the ant–seed interaction during both the pre- and post-dispersal phases and, as this study strongly supports, mice are able to considerably limit seed availability in some *H. foetidus* populations. Even though plants frequently suffer considerable impact from both pre- and post-dispersal seed predators, few studies have estimated rates of seed predation at both phases for the same plant species (Forget et al. 1999, Crawley 2000, Hulme 2002, Hulme and Benkman 2002). To our knowledge, this is the first large-scaled study that details seed predation events taking place during both phases of a same plant reproductive cycle. By doing so, it is shown that seed losses taking place at different phases can interact enhancing their overall effects and, therefore, the potential of a seed predator to constrain a plant-disperser mutualism, doing it in an enormously variable (in both space and time) manner. Consequently, we propose that to understand the true effect of antagonistic organisms on plant-animal mutualisms an approach based on multiple populations and on the whole plant's reproductive cycle may sometimes be imperative.

The relative consistency of the ant–*H. foetidus* mutualism across all scales considered here contrasted with the definitively variable incidence of mice as seed predators. This makes mice a firm candidate to constrain the ant–seed mutualism in some regions (Cazorla) whilst not in others (Caurel). Further, mice hold the potential to interfere on the development of seed traits that enable ants to efficiently harvest them, which could contribute to mosaic geographic structure on the seed–ant interaction proposed by Garrido et al. (2002). This notion is consistent with both the geographic mosaic theory of coevolution (Thompson 1994, 1999), that postulates that the form of selection between two interacting species varies across the space of coexistence, and with recent empirical evidence supporting that geographical variation in the effect of natural enemies associated with interacting species often promotes contrasting selective scenarios (Benkman 1999, Gómez and Zamora 2000, Herrera et al. 2002b, Leimu et al. 2002, Stinchcombe and Rausher 2002).

References

Alcántara, J. M., Rey, P. J., Sánchez-Lafuente, A. M. et al. 2000. Early effects of rodent post–dispersal seed predation on the outcome of the plant–seed disperser interaction. – Oikos 88: 362–370.

Armbruster, W. S. 1997. Exaptations link evolution of plant–herbivore and plant–pollinator interactions: a phylogenetic inquiry. – Ecology 78: 1661–1672.

Benkman, C. W. 1995. The impact of tree squirrels (*Tamiasciurus*) on limber pine seed dispersal adaptations. – Evolution 49: 585–592.

Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepoles pine. – Am. Nat. 153: S75–S91.

Benkman, C. W., Holimon, W. C. and Smith, J. W. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. – Evolution 55: 282–294.

Bennington, C. C. and Thayne, W. V. 1994. Use and misuse of mixed model analysis of variance in ecological studies. – Ecology 75: 717–722.

Brázdil, R. and Bíl, M. 2000. ENSO and its effects in the mean fields of air pressure, air temperature and precipitation in Europe in the 20th century. – In: Nkemdirim, L. C. (ed.), El Nino-southern oscillation and its global impacts. Commission on Climatology of the International Geographical Union, Bonn, Germany, pp. 33–62.

Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. – Ecology 78: 1624–1631.

Brown, J. H. and Davidson, D. W. 1976. Competition between seed-eating rodents and ants in desert ecosystems. – Science 196: 880–882.

Brown, J. H. and Heske, E. J. 1990. Control of a desert-grassland transition by a keystone rodent guild. – Science 250: 1705–1707.

Connell, J. H. and Green, P. T. 2000. Seedling dynamics over thirty-two years in a tropical rain forest tree. – Ecology 81: 568–584.

Crawley, M. J. 2000. Seed predators and plant population dynamics. – In: Fenner, M. (ed.), Seeds, the ecology of regeneration in plant communities. CABT Publishing, Oxford, pp. 167–182.

Edwards, G. R. and Crawley, M. J. 1999. Rodent seed predation and seedling recruitment in mesic grassland. – Oecologia 118: 288–296.

Fedriani, J. M., Delibes, M., Ferreras, P. et al. 2002. Local and landscape habitat determinants of water vole distribution in a patchy Mediterranean environment. – Ecoscience 9: 12–19.

Forget, P-M., Kitajima, K. and Foster, R. B. 1999. Pre- and post-dispersal seed predation in *Tachigali versicolor* (Caesalpiniaceae): effects of timing of fruiting and variation among trees. – J. Trop. Ecol. 15: 61–68.

Garrido, J. L., Rey, P. J., Cerdá, X. et al. 2002. Geographical variation in diaspore traits of an ant-dispersed plant (*Helleborus foetidus*): are ant community composition and diaspore traits correlated? – J. Ecol. 90: 446–455.

Gómez, J. M. and Zamora, R. 2000. Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). – Am. Nat. 155: 657–668.

Herrera, C. M. 1982. Defense of ripe fruit from pest: its significance in relation to plant-disperser interactions. – Am. Nat. 120: 218–241.

Herrera, C. M. 1984. Seed dispersal and fitness determinants in wild rose: combined effects of hawthorn, birds, mice, and browsing ungulates. – Oecologia 63: 386–393.

Herrera, C. M. 2000. Flower-to-seedling: consequences of different pollination regimes in an insect-pollinated shrub. – Ecology 81: 15–29.

Herrera, C. M., Sánchez-Lafuente, A. M., Medrano, M. et al. 2001. Geographical variation in autonomous self-pollination levels unrelated to pollinators service in *Helleborus foetidus* (Ranunculaceae). – Am. J. Bot. 88: 1025–1032.

Herrera, C. M., Cerdá, X., Garcia, M. B. et al. 2002a. Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. – J. Evol. Biol. 15: 108–121.

Herrera, C. M., Medrano, M., Rey, P. J. et al. 2002b. Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. – Proc. Natl Acad. Sc. USA 99: 16823–16828.

Hoshizaki, K. and Hulme, P. E. 2002. Mast seedling and predator-mediated indirect interactions in a forest community: evidence from post-dispersal fate of rodent-generated caches. – In: Levey, D. J., Silva, W. R. and Galetti, M. (eds), Seed dispersal and frugivory: ecology, evolution and conservation. CAB International, pp. 227–239.

Hulme, P. E. 1997. Post-dispersal seed predation and the establishment of vertebrate dispersal plants in Mediterranean scrublands. – Oecologia 111: 91–98.

Hulme, P. E. 2002. Seed-eaters: dispersal, destruction and demography. – In: Levey, D. J., Silva, W. R. and Galetti, M. (eds), Seed dispersal and frugivory: ecology, evolution and conservation. CAB International, pp. 257–273.

Hulme, P. E. and Borelli, T. 1999. Variability in post-dispersal seed predation in deciduous woodland: relative importance of location, seed species, burial and density. – Plant Ecol. 145: 149–156.

Hulme, P. E. and Hunt, M. K. 1999. Rodent post-dispersal seed predation in deciduous woodland: predator response to absolute and relative abundance of prey. – J. Anim. Ecol. 68: 417–428.

Hulme, P. E. and Benkman, C. W. 2002. Granivory. – In: Herrera, C. M. and Pellmyr, O. (eds), Plant–animal interactions. Blackwell Science, pp. 132–154.

Jaksic, F., Silva, S., Meserve, P. et al. 1997. A long-term study of vertebrate predator responses to an El Niño (ENSO)

disturbance in western South America. – Oikos 78: 341–354.

Janzen, D. H. 1971. Seed predation by animals. – Annu. Rev. Ecol. Syst. 2: 465–492.

Kollmann, J., Coomes, D. A. and White, S. M. 1998. Consistencies in post-dispersal seed predation of temperate fleshy-fruited species among seasons, years and sites. – Funct. Ecol. 12: 683–690.

Littell, R. C., Milliken, G. A., Stroup, W. W. et al. 1996. SAS system for fixed models. – SAS Institute.

Leimu, R., Syrjänen, K., Ehrlén, J. et al. 2002. Pre-dispersal seed predation in *Primula veris*: among-population variation in damage intensity and selection on flower number. – Oecologia 133: 510–516.

Lord, J. M., Markey, A. S. and Marshall, J. 2002. Have frugivores influenced the fruit traits evolution in New Zealand? – In: Levey, D. J., Silva, W. R. and Galetti, M. (eds), Seed dispersal and frugivory: ecology, evolution and conservation. CAB International, pp. 55–68.

Rey, P. J., Garrido, J. L., Alcántara, J. M. et al. 2002. Spatial variation in ant and rodent post-dispersal predation of vertebrate-dispersed seeds in southern Spain. – Funct. Ecol. 16: 773–781.

Sallabanks, R. 1993. Hierarchical mechanisms of fruit selection by an avian frugivore. – Ecology 74: 1326–1336.

Schabenberger, O., Gregoire, T. G. and Kong, F. 2000. Collections of simple effects and their relationships to main effects and interactions in factorial. – Am. Stat. 54: 210–214.

Smith, C. C. and Reichman, O. J. 1984. The evolution of food caching by birds and mammals. – Annu. Rev. Ecol. Syst. 15: 329–351.

Stinchcombe, J. R. and Rausher, M. D. 2002. The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. – Proc. R. Soc. Lond. (B) 269: 1241–1246.

Thompson, J. N. 1994. The coevolutionary process. – Univ. of Chicago Press.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. – Am. Nat. 153 (suppl.): S1–S14.

Thompson, J. N. and Pellmyr, O. 1992. Mutualism with pollinating seeds parasites amid co-pollinators: constraints on specialization. – Ecology 73: 1780–1791.

Vander Wall, S. B. 1990. Food hoarding in animals. – Univ. of Chicago Press.

Vander Wall, S. B. 2002. Masting in animal-dispersed pines facilitates seed dispersal. – Ecology 83: 3508–3516.

Vander Wall, S. B. 2003. Effects of seed size of wind-dispersed pine s (*Pinus*) on secondary seed dispersal and the caching behavior of rodents. – Oikos 100: 25–34.

Wang, B. C. and Smith, T. B. 2002. Closing the seed dispersal loop. – Trends Ecol. Evol. 17: 379–385.

Wenny, D. G. 2000. Seed dispersal, seed predation, and seedling recruitment of a neotropical montane tree. – Ecology 70: 331–351.

Werner, K. and Ebel, F. 1994. Zur Lebensgeschichte der Gattung *Helleborus* L. (Ranunculaceae). – Flora 189: 97–130.

Willson, M. F. and Whelan, C. J. 1990. Variation in post-dispersal survival of vertebrate-dispersed seeds: effects of density, habitat, location, season and species. – Oikos 57: 191–198.

Zamora, R. 2000. Functional equivalence in plant-animal interactions: ecological and evolutionary consequences. – Oikos 88: 442–447.