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A multi-scale approach in the study of plant regeneration: Finding bottlenecks is not enough

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Abstract

Using a multi-scale design, we investigated the spatio-temporal variability and concordance across recruitment stages of the Mediterranean fleshy-fruited shrub *Daphne gnidium*. The patterns of seed rain, post-dispersal seed predation, seedling emergence, survival and growth were studied in two populations in contrasting conditions, in an olive grove and in a dune system, in Mallorca (Balearic Islands) during two consecutive seasons. We considered 20 sites (fine scale) in four microhabitats (medium scale) in each population (large scale). Most seeds were dropped under parent plants, especially in the population with presumably less seed dispersers. Post-dispersal seed predation (ca. 90%) represented the most important bottleneck in plant recruitment in all microhabitats at both populations. Seedling emergence was also similar at both populations although seedling survival was higher in the olive grove. One year after of emergence, seedlings under shrubs were taller – though not necessarily had more leaves – than in the other microhabitats. There was a positive and consistent effect of seed size across microhabitats on seedling emergence, survival and growth (maximum length) although its intensity varied between populations and years. Concordance between seed rain and seedling recruitment was affected by the heterogeneity of population-dependent forces acting upon each recruitment stage. There were stages, which represented a strong bottleneck but were little discordant with seed rain. By contrast, other weak bottlenecks were hardly discordant with seed rain. Our study stresses the importance of considering simultaneously the critical stages (i.e., bottlenecks) and the concordance between seed dispersal and post-dispersal stages in plant recruitment studies.
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Introduction

The plant recruitment process is considered a sum of linked stages that are proportional and sequentially affecting the final regeneration of a given species (Clark et al., 1999). An increasing but still low number of studies have considered this linkage among stages (Jordano and Herrera, 1995; Lázaro et al., 2006; Rey and Alcántara, 2000; Traveset et al., 2003), assessing the importance of each one and identifying those that represent bottlenecks (i.e., those stages that critically affect plant recruitment).

The first of this series of stages is seed dispersal, which determines the spatial arrangement (and consequently the biotic and abiotic template for further stages) of a

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new cohort of seedlings (for a review, see Nathan and Muller-Landau, 2000). In biotically dispersed plants, the behaviour, preferences and abundance of the animal seed dispersers can influence further stages because they directly affect the processes of fruit removal and seed deposition (Russo and Augspurger, 2004; Schupp et al., 2002; Wenny and Levey, 1998) and consequently influence the spatial and the genetic structure of plant recruitment (reviewed by Levin et al., 2003). The recruitment process is also importantly influenced by the abiotic conditions and environmental resources prevailing in the habitat where the plant lives (Clark et al., 1999; Fowler, 1988; Gómez-Aparicio et al., 2005). In Mediterranean ecosystems, in particular, summer drought is usually the main cause of seedling mortality (e.g., Giménez-Benavides et al., 2007; Jordano and Herrera, 1995; Rey and Alcántara, 2000; Traveset et al., 2003), and differences in seedling survival in them strongly depend on the abundance of safe microsites (e.g., nurse plants), which in turn are influenced by the climatic conditions of each population and year (Brooker, 2006; Callaway et al., 1996; Fowler, 1988). In the case of fleshy-fruited plants, such facilitation effect is enhanced as most seeds are deposited under shrubs (perches), which tend to be the most successful microhabitat for seedling recruitment (Wenny, 2001). Indeed, the effect of nurse plants seems to be crucial in arid ecosystems because they ameliorate the negative effect of summer drought in seedling survival, at least when resources are limiting (Maestre et al., 2005).

A spatial discordance of stages at the level of microhabitat has been hypothesized to be inherent to plant recruitment (Bazzaz, 1991; Houle, 1998; Jordano and Herrera, 1995; Schupp, 1995; Wenny, 2000). Such discordance results from each stage being subjected to different forces (i.e., seed dispersers, seed predators or micro-climate) that are intrinsically heterogeneous in space and time (Schupp, 1995; Wenny, 2000). Besides determining this heterogeneity, however, it is also important to detect how variable those stages are and how this variation is shared through the recruitment process, because the concordance between seed rain and post-dispersal stages depends on the relative variability across them (García et al., 2005; Kollmann, 2000). Moreover, when determining the relative importance of each stage through the recruitment process and how it varies across microhabitats (Castro et al., 2004; Gómez-Aparicio et al., 2004; Gulías et al., 2004; Lázaro et al., 2006; Traveset et al., 2003), it is also relevant to understand the contribution of subsequent scales to the total variance of a given stage (Kollmann, 2000).

Seed size is another possible source of variability in the plant recruitment process that has not received much attention so far. Despite the large number of studies examining the consequences of seed size in different fitness components, there is actually scarce information

on how variability in this trait affects the different recruitment stages (Alcántara and Rey, 2003; Gómez, 2004; A. Lázaro, unpublished data) and how such influence can vary at different spatial and temporal scales (e.g., Rey et al., 2004).

In the present study, our goal was to determine the critical stages on the recruitment of a Mediterranean fleshy-fruited shrub, *Daphne gnidium* L., at the same time that we evaluate at different spatial (site, micro-habitat and population) and temporal (annual) scales, the variability and concordance across stages. We further investigated how the variability in seed size may alter the outcome at each scale. Two populations in Mallorca (Balearic Islands; W. Mediterranean) with contrasting climatic conditions were selected. Our specific questions were: (a) How important and variable are the recruitment stages under different spatial scales? (b) Does seed size have an influence across stages – from seedling emergence to growth – and how does this influence vary among different scales? (c) Is there concordance (at the level of scale) between seed deposition patterns and the rest of post-dispersal stages? (d) When predicting the patterns of plant recruitment, is the magnitude of loss of a recruitment stage (i.e., bottleneck) more important than concordance between seed dispersal and the subsequent stages?

Material and methods

Study species and sites

Daphne gnidium L. (Thymelaeaceae; *Daphne* hereafter) is an evergreen shrub, up to 1.5 m tall, which distribution is restricted to the Mediterranean basin and Canary Islands. Its flowers are hermaphroditic, tubular and cream coloured, and have a sweet smell during the day. The fruits, red-orange drupes dispersed mostly by birds (Herrera, 1987), are ripe from October to December. Each shrub produces an average of ca. 250 fruits. The species shows a clear pattern of nucleation associated to a perching effect by birds (Verdú and García-Fayos, 1998).

One of the study populations is located at the north-western coast of Mallorca, near the village of Deià (2°38′E, 39°44′N; 500 a.s.l.; 'olive grove' population hereafter). The site is an abandoned olive grove (*Olea europaea* var. *europaea*), which is currently being recolonized by natural vegetation, mainly *Pinus halepensis*, *Pistacia lentiscus*, *Ampelodesmos mauritatica* and *Erica multiflora*. *Daphne* plants are mainly located under the canopy of olive trees. The other population occurs at the northern part of the island, in a coastal shrubland located on fixed dunes near Ca'n Picafort (3°08′E, 39°46′N; 10 a.s.l.; 'dune' population hereafter). The predominant vegetation here consists of *E. multiflora*,

Halimium halimifolium, *P. lentiscus*, *Cistus salvifolius*, *Rosmarinus officinalis* and *Thymelaea velutina*. *Daphne* plants here are usually found in clearings (open interspaces).

Sampling design

Three subsequent and hierarchical spatial levels ('site', 'microhabitat' and 'population') and 2 years were considered in order to determine the spatial and temporal variability of each recruitment stage in *Daphne*. Site is here the finest grain of spatial hetero-geneity, accounting for less than $20\,m^2$. Microhabitat represents physiognomically distinguishable patches of vegetation (or no vegetation) types that are common at a higher scale ($100–500\,m^2$). Lastly, population accounts for differences occurring at a regional scale ($>1\,km$), with important differences in their vegetation structure and resource availability.

In each population, we selected a total of 20 sites, which contained a representative of the four major groups of microhabitats. We thus had a total of 80 'locations' (sampling unit) per population. The four locations in each site were placed less than $5\,m$ away from each other but at least $10\,m$ away from the nearest site. The microhabitats considered in both populations were (1) 'under *Daphne*', (2) 'open interspaces' ('open', hereafter) and (3) 'under *P. lentiscus*', a fleshy-fruited shrub that fruits simultaneously with *Daphne* ('under *Pistacia*' hereafter). The fourth microhabitat varied in the two populations: it was 'under *O. europaea*' ('under *Olea*' hereafter) in the abandoned olive grove and 'under *E. multiflora*' ('under *Erica*' hereafter) in the dune. The potential reproductive losses at each stage of the recruitment process were measured in each location.

We determined the abundance of each microhabitat by performing $20\,m$ long vegetation transects ($n = 12$) in each population. In each transect, we haphazardly took 12 start points, assigning a random bearing of a compass to each one and measuring the percentage of each plant species that crossed a measuring tape.

Seed rain

Seed dispersal was assessed during the autumns of 2003 and 2004. Additional data on seed rain was obtained in 2005, although this cohort of seeds was not followed during the further stages. We began monitoring seed rain as soon as *Daphne* fruits were ripe in each population, from early October to mid November. Sampling was every 10 days and was maintained until no fruits remained on plants. Each fruit was considered dispersed when the seed was found without pulp. Seeds and fruits were collected in aluminium trays of $0.040\,m^2$ that were covered with a

grid ($2\,cm$ wide) to prevent predation by vertebrates. A tray was placed in each location. Since *Daphne* plants in the olive grove were mostly located under the canopy of olive trees, a small fraction of dispersed fruits deposited 'under *Daphne*' might have eventually been defecated by a bird perching on olive trees. Some *Daphne* individuals of the dune population, whose fruits were collected in 2003, did not produce fruits in 2004 and 2005, so we replaced them with new ones located within the study area.

Post-dispersal seed predation

We examined post-dispersal seed predation in the reproductive seasons of 2003 and 2004. Fresh drupes were collected from plants more than $100\,m$ outside the experimental area of each population. We considered fruits instead of seeds because most fruits fall without being dispersed (see results). Each sampling unit consisted of a pair of fruit traps ($15\,cm \times 15\,cm$, made of plastic net of $2\,mm$ light) with 10 fruits each. By means of a wire mesh ($1\,cm \times 1\,cm$ grid), half of the traps (one from each pair) were excluded to vertebrate predators (mostly the rodent *Apodemus sylvaticus*, although eventual activity of granivorous birds inhabit-ing the nearby forests cannot be ruled out) whereas the other half was left as controls (i.e., exposed to vertebrates and ants). In 2004, the low fruit production in the dune made us to reduce by half the number of sites ($n = 10$) used for this experiment (and for the subsequent stages) in that population. The number of intact fruits remaining in the traps was recorded after 2, 10 and 40 days of setting the experiment. We considered seed removal to be almost equivalent to seed predation, although we cannot discard the possibility of some secondary dispersal (see limitations of this assumption in Vander Wall et al., 2005). We justify our assumption because (1) we did not observe any active ant species in the study areas when most fruits were available (winter time), (2) we never observed intact seeds in the ant nests found and (3) seed remains typical of rodent predation were often found in the fruit traps.

Seedling emergence and survival

In 2003 and 2004, we collected fruits in a total of eight plants in each population and obtained pulp-free seeds to be sown in the field (each group of seeds to each respective population). Owing to the large annual variation in fruit production within an individual, the source plants were not the same the two study years. Each fruit was manually depulped in the laboratory and the seed was weighted to the nearest $0.01\,mg$. We sowed 16 seeds per location, at a depth of ca. $0.5\,cm$ and in an area of $15\,cm \times 10\,cm$. Two seeds of each mother plant

were included in the experimental unit in order to control for maternal variability. Seed sowing took place by mid January of each year following collection, i.e., few weeks after dispersal finished.

Before the summer period, seedling emergence was checked three times in 2004 and twice in 2005, whereas seedling survival was recorded in November both years. At this time, we also recorded two measures of seedling growth: length (to the nearest mm) and number of leaves. Seedling mortality was assigned to the most evident cause (a) 'pathogens' when seedlings died shortly after emergence, (b) 'herbivory' when all leaves or cotyledons were eaten and (c) 'summer drought' when seedlings were dry after the summer period.

Cumulative probability of recruitment

We calculated four transition probabilities (TP) between different stages from seed dispersal to seedling establishment, similarly to what has been done in other studies (e.g., Gulías et al., 2004; Lázaro et al., 2006; Rey and Alcántara, 2000; Traveset et al., 2003). The TPs were the following: TP_1: probability of seed deposition, TP_2: of escaping seed predation, TP_3: of seedling emergence and TP_4: of seedling survival after the first summer. The cumulative probability of recruitment (CPR) was calculated as the product of all TPs for each population, habitat and year.

An overall probability of recruitment (OPR) was estimated for each population as the product of CPR by the proportion of the area occupied by each microhabitat. Besides the four microhabitats considered in each locality, a fifth category included 'the rest of microhabitats', i.e., mainly other dry-fruited shrubs and herb species, due to the low seed rain occurring in these microhabitats, this category was finally pooled with the open microhabitat for the calculation of OPR.

Data analyses

Statistical analyses were performed using generalized linear models (GLIMs) and likelihood ratio test III, with the GENMOD procedure of SAS 9.0 statistical package (SAS Institute, 2000). A repeated measures design was used for most analyses, including 'site' as a subject random factor and 'microhabitat', 'population' and 'year' as within-subject fixed factors. The analyses compared the three common microhabitats shared by both populations. Additional analyses, which included the four microhabitats, were carried out for each population separately. In all models, we considered the distribution and link function that minimized their deviance.

Seed rain data were analysed by performing two different tests: one considered the presence/absence of

Daphne seeds in the traps (using a binomial distribution and a logit link function), and the other the proportion of dispersed seeds (number of dispersed seeds relative to all seeds), using a normal distribution and a log link function. For the dune population, the first test considered only the 'under *Daphne*' microhabitat, as all fruits fell under conspecific plants (see results). Seed predation, seedling emergence and survival data were also fitted to binomial distributions and a logit link function whereas both variables of seedling growth were adjusted to a gamma distribution and a log link function. Seed mass was included as a covariate in the seed predation and seedling emergence models, after testing for homogeneity of slopes and excluding those effects with $p > 0.25$ (Quinn and Keough, 2002). For seedling survival and growth, we further tested the effect of seedling density by repeating the analyses, this time considering the average of seedling survival and growth in each location as the dependent variables and including the number of seedlings for each experimental location as a covariate. Data on CPR at each site were adjusted to a normal distribution and a log link function.

Variance components at the different spatial and temporal scales for each stage were evaluated using GLIMs and likelihood ratio test I. Site was nested within microhabitat, which in turn was nested within population (two microhabitats were exclusive of each population). Models were fitted to the same distributions and link functions as before. Deviance of each factor was used to calculate the variance percentage at each scale.

Means are consistently accompanied by their standard errors (mean ± SE) throughout the test unless otherwise indicated.

Results

Seed dispersal

Ripen fruits remained only a short time (about 3–4 days) on the branches, being dropped underneath their parent plants, which reduces their probability of being dispersed by birds. The probability of seed deposition in the different microhabitats varied between populations (Table 1). In the olive grove, the greatest proportion of seeds (92.1 ± 4.0%) felt under conspecifics, whereas much smaller fractions were deposited under *Olea* (6.3 ± 3.3%) or *Pistacia* (1.6 ± 1.6%), and no seeds at all in the open interspaces (the last three microhabitats did not differ among them, Table 1). In the dune, despite the larger number of fruits falling in trays compared with the olive grove (12.6 vs. 3.2 fruits/tray on average, respectively), all seeds were located under conspecifics

Table 1. Results of the generalized linear model showing differences between years, microhabitats for seed dispersal and post-dispersal seed predation in *Daphne gnidium* by each population separately

Recruitment stage	Analysis	Effect	d.f.	χ^2
Seed dispersal	Olive grove	Microhabitat	3	114.1***
		Year	2	29.27***
	Dune	Year	2	5.52†
	Both populations	Microhabitat	2	277.6***
		Year	2	44.23***
		Population	1	2.68n.s.
Post-dispersal seed predation	Olive grove	Microhabitat	3	5.56n.s.
		Year	1	4.05*
		Exclusion	1	19.73***
		Mhab × excl	3	1.02n.s.
		Mhab × year	3	1.88n.s.
		Year × excl	1	0.50n.s.
	Dune	Microhabitat	3	5.02n.s.
		Year	1	1.72n.s.
		Exclusion	1	13.92**
		Mhab × excl	3	3.84n.s.
		Mhab × year	3	4.42n.s.
		Year × excl	1	1.92n.s.
	Both populations	Microhabitat	2	1.86n.s.
		year	1	1.68n.s.
		Exclusion	1	27.37***
		Population	1	0.13n.s.
		Mhab × excl	2	1.18n.s.
		Mhab × year	2	2.69n.s.
		Mhab × pop	2	2.27n.s.
		Year × excl	1	1.02n.s.
		Year × pop	1	3.41†
		Pop × excl	1	1.00n.s.

The population effect was tested in separate analyses, including only the common microhabitats in both populations. For the seed dispersal stage, two-way interactions were not included in the final model. Vertebrate exclusion effect was included in post-dispersal seed predation analyses. Three- and four-way interactions were not shown as they were non-significant ($p \gg 0.05$).
Significance: *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, †$p < 0.1$, n.s. $p > 0.1$.

during the three monitored years, which indicated almost nil dispersal in that population. Moreover, when considering only the proportion of defecated seeds falling under *Daphne* – relative to the total number found in trays – it was much greater in the olive grove than in the dune ($42.7 \pm 6.7\%$ vs. $4.85 \pm 1.62\%$, respectively; $F_{2,29} = 9.16$, $p = 0.005$); annual differences on such proportion were only marginal ($F_{2,29} = 3.29$, $p = 0.051$). The probability of seed dispersal differed among years in both populations (Table 1), being highest in 2003 ($35.8 \pm 4.4\%$), lowest in 2004 ($15.8 \pm 9.2\%$), and intermediate in 2005 ($25.6 \pm 4.1\%$). Such variation was greater in the olive grove than in the dune.

Post-dispersal seed predation

The levels of post-dispersal seed predation were quite high in both populations: 40 days after setting the experiment, only an average of $9.0 \pm 2.3\%$ of the seeds remained in the traps. Such predation was similarly intense in all microhabitats, years and populations (Table 1). In both populations, seeds were mostly preyed upon by vertebrates, as up to $90.9 \pm 1.7\%$ of the seeds remained in the vertebrate-exclusion trays (Table 1). Given the signs observed (opened seeds, with no embryo and only the testa remaining), seeds were presumably consumed by rodents (mostly wood mice, *A. sylvaticus*, though *Rattus* spp. is not discarded); some invertebrates might also slightly contribute to seed removal and/or predation.

Seedling emergence and recruitment

The majority (93%) of seeds sown in January germinated before mid-April (both populations and years pooled). Marginal differences among microhabitats in seedling emergence were found only in the dune

Table 2. Temporal and spatial variation in the effect of seed mass on seedling emergence and survival in *Daphne gnidium*

Recruitment stage	Analysis	Effect	d.f.	χ^2
Seedling emergence	Olive grove	Microhabitat	3	4.96n.s.
		Year	1	5.35*
		Mhab × year	3	2.21n.s.
		Seed mass	1	6.97*
		Sdm × Mhab	3	5.28n.s.
		Sdm × year	1	8.86*
	Dune	Microhabitat	3	7.01†
		Year	1	5.61*
		Mhab × year	3	3.92n.s.
		Seed mass	1	11.07**
		Sdm × year	1	5.92*
	Both populations	Microhabitat	2	1.13n.s.
		Year	1	8.92*
		Population	1	18.12***
		Mhab × year	2	3.16n.s.
		Mhab × pop	2	8.03*
		Year × pop	2	5.31*
		Seed mass	1	24.81***
		Sdm × Mhab	2	3.25n.s.
		Sdm × year	1	11.34**
		Sdm × pop	1	10.45*
Seedling survival	Olive grove	Microhabitat	3	7.31†
		Year	1	15.13***
		Mhab × year	3	5.67n.s.
		Seed mass	1	0.05n.s.
	Dune	Microhabitat	3	11.74*
		Year	1	2.71†
		Mhab × year	3	1.92n.s.
		Seed mass	1	6.00*
	Both populations	Microhabitat	2	11.73*
		Year	1	8.67*
		Population	1	1.81n.s.
		Mhab × year	2	1.47n.s.
		Mhab × pop	2	10.28*
		Year × pop	1	1.78n.s.
		Seed mass	1	3.59†
		Sdm × pop	1	1.79n.s.

As occurred in Table 1, the population effect was tested in separate analyses. Three- and four-way interactions were not included due to lack of significance ($p \gg 0.05$).
Significance symbols as in Table 1.

and only when pooling years (Table 2 and Fig. 1), being highest 'under *Pistacia*' (55.4±4.3%) and lowest in the open (37.7±3.5%). In general, seedling emergence was greater in the olive grove (68.6±1.5%) than in the dune (46.7±2.4%, Table 2), and this was consistent during the two years (Fig. 1). Considering the intra-population variation, seedling emergence was significantly lower in 2003 than in 2004 in the olive grove (62.7±2.1% vs. 73.9±2.0%) while the opposite occurred in the dune (48.8±2.7% vs. 42.0±3.8%, Table 2).

Seedling emergence was more likely for large than for small seeds in both populations (Table 2). A highly significant interaction between seed mass and

population was found (Table 2), as the effect of seed mass was stronger in the dune [$\log_e(p/1-p) = 0.34x - 2.72$] than in the olive grove [$\log_e(p/1-p) = 0.18x - 0.66$]. The effect of seed mass on seedling emergence did not vary among microhabitats and was fairly consistent between years in the two populations (Table 2).

Summer drought was the major cause of seedling mortality (83.2%, both years and populations pooled), followed by pathogens (6.5%) and herbivory (3.2%). There were no differences between populations in seedling survival (Table 2), but as in the case of seedling emergence, differences among microhabitats were larger
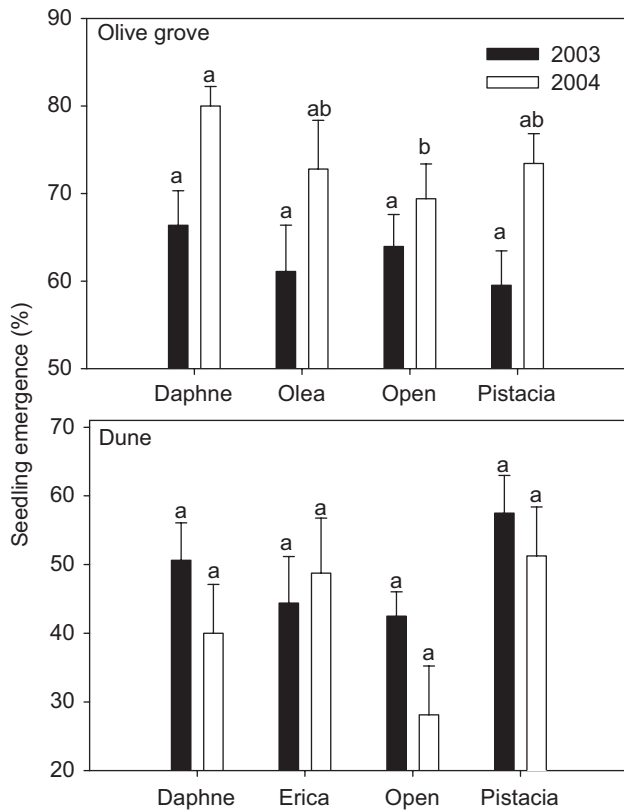
Fig. 1. Proportion of seedling emergence (± 1 SE) in *Daphne gnidium*, for each population, year and microhabitat. Bars with the same letter do not differ significantly among microhabitats ($p < 0.05$), for each population and year.
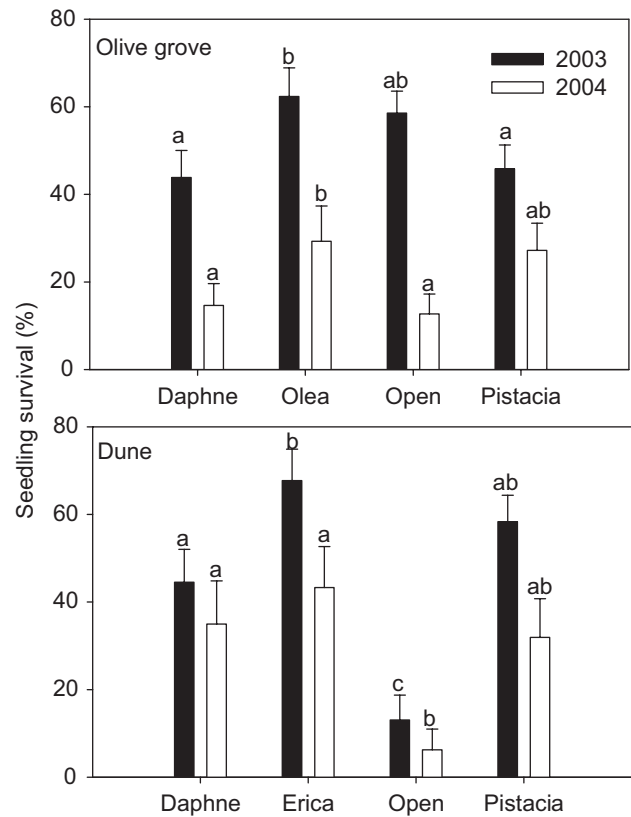

Fig. 2. Proportion of seedling survival (± 1 SE) in *Daphne gnidium*, for each population, year and microhabitat. Other symbols as in Fig. 1.

in the dune than in the olive grove (Table 2). In the former, the lowest seedling survival occurred in the open ($11.1 \pm 4.3\%$, both years pooled) and there were no differences among the other microhabitats (Fig. 2). By contrast, the lowest seedling survival in the olive grove was under *Daphne* ($29.6 \pm 4.6\%$) whilst the highest was under *Olea* ($46.2 \pm 5.8\%$). Seedling survival did not vary between years in the dune, but it was greater in the olive grove in 2003 ($52.6 \pm 3.0\%$) than in 2004 ($21.0 \pm 3.1\%$; Table 2). Seed mass positively influenced seedling survival, but only in the dune, and did not vary between years or among microhabitats (Table 2). Moreover, seedling survival was not affected by density of emerged seedlings ($\chi^2 = 1.71$, d.f. $= 1$, $p = 0.164$).

Seedling growth

Results on seedling growth differed depending upon whether the variable considered was seedling length or number of leaves per seedling (Fig. 3). Thus, microhabitat had a significant influence on seedling length at both populations (olive grove: $\chi^2 = 9.21$, d.f. $= 3$, $p = 0.028$; dune: $\chi^2 = 9.57$, d.f. $= 3$, $p = 0.023$), but not on the number of leaves ($\chi^2 = 5.46$, d.f. $= 3$,

$p = 0.141$, for either population). In the olive grove, although there were differences among years in seedling length, seedlings growing in the open were consistently smaller than in the rest of microhabitats. In the dune, seedlings were longest under *Pistacia* and shortest in the open (Fig. 3). Overall, seedlings were longer in the olive grove (68.3 ± 3.2 mm) than in the dune (45.4 ± 6.6 mm; $\chi^2 = 4.32$, d.f. $= 1$, $p = 0.038$), although they produced similar amounts of leaves in both populations ($\chi^2 = 1.07$, d.f. $= 1$, $p = 0.300$). Seedlings grew more in 2004 than in 2003 in the olive grove ($\chi^2 = 4.34$, d.f. $= 1$, $p = 0.037$) but not in the dune ($\chi^2 = 0.08$, d.f. $= 1$, $p = 0.783$), while no differences were found between years to the number of leaves ($\chi^2 < 1.84$, d.f. $= 1$, $p > 0.175$, for either population).

Heavier seeds tended to produce longer seedlings ($\chi^2 = 6.81$, d.f. $= 1$, $p = 0.009$, for both populations), both in the olive grove [$\log_e(p) = 0.04x + 3.29$] and in the dune [$\log_e(p) = 0.04x + 3.07$]; at least in the former, this seed mass effect varied temporally, and it was significantly stronger in 2003 than in 2004 ($\chi^2 = 4.75$, d.f. $= 1$, $p = 0.029$). By contrast, seed mass was not associated to number of leaves per seedling and did not covary with microhabitat, year or population ($p \gg 0.05$ for all effects). Finally, seedling density had no significant
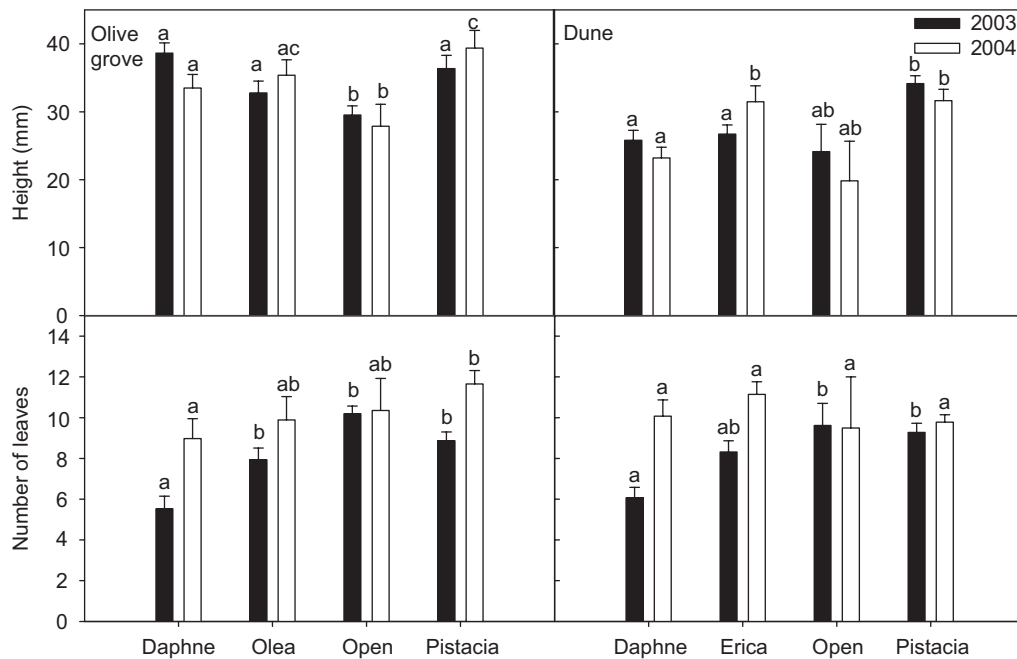
Fig. 3. Seedling growth (maximum length and number of leaves, ± 1 SE) in *Daphne gnidium*, for each population, year and microhabitat. Other symbols as in Fig. 1.

influence on either seedling length or number of leaves ($\chi^2 < 0.03$, d.f. $= 1$, $p > 0.862$, for either variable).

Cumulative probability of recruitment

CPR differed significantly among microhabitats, populations, and years, and there were significant interactions among the three factors ($\chi^2 > 200.9$, $p < 0.0001$, for all effects). It was lower in the olive grove than in the dune during 2003 (4.26×10^{-5} and 2.85×10^{-4}, respectively) but the opposite was found the following year (1.67×10^{-4} and 8.33×10^{-5}, respectively). Moreover, while the microhabitat with the highest probability of seedling establishment was under *Daphne* in both populations, this was not temporally consistent, as in 2004 the olive grove showed similar CPR values under *Daphne* as under *Olea* (Fig. 4).

When calculating OPR, which considered the area occupied by each microhabitat, we found that the large abundance of 'open' microhabitat in the olive grove compensated the low capacity of recruitment and, consequently, OPR was highest there, but only in 2003 (Fig. 4). In 2004, a dispersed seed had a ten-fold higher probability of recruitment under an olive tree than under a conspecific. In the dune, by contrast, OPR was higher under *Daphne* and this was consistent through time (Fig. 4).

Scale variability across recruitment stages

Despite most variance was explained at the site ($=$ fine spatial) scale, variance partitioning depended

upon each recruitment stage (Table 3). Thus, for instance, variance in seed rain and seedling emergence was low at the microhabitat scale but high at the population level (Table 3). On the contrary, a high and low variability at the microhabitat and population levels, respectively, were detected for both post-dispersal seed predation and seedling survival. Regarding seedling growth (length, in particular) no differences among scales were observed (i.e., variance was equally partitioned across scales). On the other hand, temporal (annual) variability was especially important in some stages such as seed rain, seedling survival and seedling growth (number of leaves), being in some cases higher than the variability at the microhabitat scale (Table 3). Finally, the effect of seed mass was especially variable in some stages, such as seedling emergence and growth (Table 3).

Discussion

Stages acting as bottlenecks in plant recruitment

There are important differences in the magnitude of reproductive losses of the stages affecting the recruitment of *Daphne*. Firstly, seed dispersal showed to especially affect the spatial structure of this plant, as seed arrival is quite limiting in microhabitats other than under conspecifics. This appears to be a consequence of the low fruit consumption by birds (Herrera, 1998). Independently of conspecific adult coverage (ca. 4% of space occupancy of *Daphne* in both populations; see
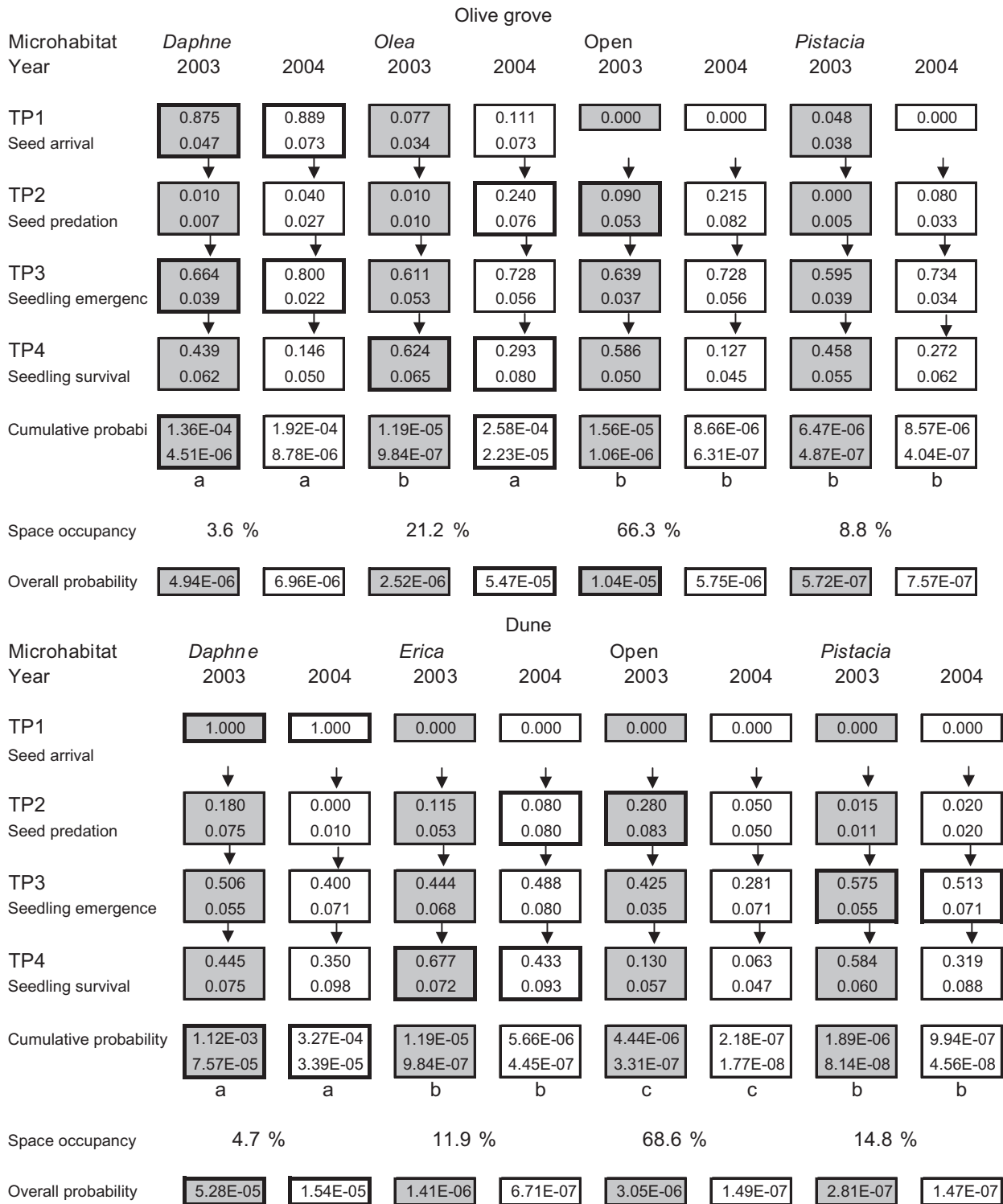
Olive grove

Microhabitat	*Daphne*		*Olea*		Open		*Pistacia*	
Year	2003	2004	2003	2004	2003	2004	2003	2004
TP1 Seed arrival	0.875 0.047	0.889 0.073	0.077 0.034	0.111 0.073	0.000	0.000	0.048 0.038	0.000
TP2 Seed predation	0.010 0.007	0.040 0.027	0.010 0.010	0.240 0.076	0.090 0.053	0.215 0.082	0.000 0.005	0.080 0.033
TP3 Seedling emergenc	0.664 0.039	0.800 0.022	0.611 0.053	0.728 0.056	0.639 0.037	0.728 0.056	0.595 0.039	0.734 0.034
TP4 Seedling survival	0.439 0.062	0.146 0.050	0.624 0.065	0.293 0.080	0.586 0.050	0.127 0.045	0.458 0.055	0.272 0.062
Cumulative probabi	1.36E-04 4.51E-06	1.92E-04 8.78E-06	1.19E-05 9.84E-07	2.58E-04 2.23E-05	1.56E-05 1.06E-06	8.66E-06 6.31E-07	6.47E-06 4.87E-07	8.57E-06 4.04E-07
	a	a	b	a	b	b	b	b
Space occupancy	3.6 %		21.2 %		66.3 %		8.8 %	
Overall probability	4.94E-06	6.96E-06	2.52E-06	5.47E-05	1.04E-05	5.75E-06	5.72E-07	7.57E-07

Dune

Microhabitat	*Daphne*		*Erica*		Open		*Pistacia*	
Year	2003	2004	2003	2004	2003	2004	2003	2004
TP1 Seed arrival	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
TP2 Seed predation	0.180 0.075	0.000 0.010	0.115 0.053	0.080 0.080	0.280 0.083	0.050 0.050	0.015 0.011	0.020 0.020
TP3 Seedling emergence	0.506 0.055	0.400 0.071	0.444 0.068	0.488 0.080	0.425 0.035	0.281 0.071	0.575 0.055	0.513 0.071
TP4 Seedling survival	0.445 0.075	0.350 0.098	0.677 0.072	0.433 0.093	0.130 0.057	0.063 0.047	0.584 0.060	0.319 0.088
Cumulative probability	1.12E-03 7.57E-05	3.27E-04 3.39E-05	1.19E-05 9.84E-07	5.66E-06 4.45E-07	4.44E-06 3.31E-07	2.18E-07 1.77E-08	1.89E-06 8.14E-08	9.94E-07 4.56E-08
	a	a	b	b	c	c	b	b
Space occupancy	4.7 %		11.9 %		68.6 %		14.8 %	
Overall probability	5.28E-05	1.54E-05	1.41E-06	6.71E-07	3.05E-06	1.49E-07	2.81E-07	1.47E-07

Fig. 4. Diagram of the spatial and temporal dynamics of the recruitment of *Daphne gnidium*. The mean of the transition probabilities (± 1 SE, i.e., the probability that each seed or seedling passes to the subsequent stage) for each population, year and recruitment stage (seed rain, post-dispersal seed predation, seedling emergence and first year survival). A thicker line around the box reflects a greater probability of this microhabitat for a given stage, each population and year. Boxes (CPR) with the same letter did not differ significantly ($p < 0.05$) among microhabitats, for each population and year. Whenever a stage in a given population, year or microhabitat performed a null value ($p = 0.000$), we considered a probability of $p = 0.001$ in order to be able to calculate the CPR. Figure based on Fig. 2 in Rey and Alcántara (2000).

Table 3.   Percentage of the relative variance across the different regeneration stages of *Daphne gnidium* in different spatio-temporal scales (main factors) and also including seed mass for seedling emergence, survival and growth

	Site	Microhabitat	Population	Year	Seed mass
Seed rain	30.6	1.0	32.3	36.1	–
Seed predation	68.6	28.1	1.0	2.4	–
Seedling emergence	37.2	7.8	38.6	1.3	15.1
Seedling survival	35.4	26.4	1.4	36.0	0.8
Seedling growth (length)	41.0	25.4	24.4	0.0	9.2
Seedling growth (*N* leaves)	47.7	13.5	0.3	34.8	3.7
Mean	43.4	17.0	16.3	18.4	7.2

Relative variance was calculated from generalized linear models, considering site nested within microhabitat and microhabitat nested within population.

Table 3), dispersal of *Daphne* seeds was consistently higher in the olive grove that in the dune which we attribute to differences in frugivore abundance: although no frugivore censuses were performed, we occasionally observed blackcaps (*Sylvia atricapilla*) and European robins (*Erithacus rubecula*) visiting *Daphne* shrubs in the olive grove whilst never observed birds on *Daphne* in the dune. Secondly, post-dispersal seed predation was the most important bottleneck in both populations. Such high seed density (ca. 444 seeds/m^2) in experimental conditions might be overestimating seed predation to some extent in the field as it may promote a higher detectability of sample units by seed predators. Nevertheless, the lack of differences in seed predation among microhabitats indicated that predators (mostly rodents) are unlikely to change the seed deposition patterns. Thirdly, seedling emergence was relatively high (ca. 60%) and, thus, it is unlikely that this stage represents an important bottleneck. Finally, a relevant fraction (ca. 40%) of those emerged seedlings recruited during the first year and survived the second year (ca. 70%). As occurs for most Mediterranean shrubs (e.g., Castro et al., 2004; Gulías et al., 2004; Giménez-Benavides et al., 2007; Lázaro et al., 2006; Rey and Alcántara, 2000; Traveset et al., 2003), summer drought was the most important cause of seedling mortality in *Daphne* (>80%).

The lack of seed dispersal produces a high seed density under the parent plants and, thus, we might expect a negative density-dependent effect on seedling fitness due to intraspecific competition for resources (Nathan and Muller-Landau, 2000). In the present case, however, we found only a slightly lower seedling survival and/or growth under *Daphne* than under other shrubs, and detected no significant effect of seedling density on either dependent variable. Hence, at least in the short term, a high density of seedlings underneath the mother plant might not limit plant recruitment (Stevenson, 2007), as far as those mothers could be substituted by their offspring. In this study, we did not compare the fate of dispersed vs. undispersed seeds and,

thus, we cannot assure that the low or lack of dispersal limits plant recruitment (Robertson et al., 2006). However, the also rather high survival in the microhabitats, except in the open, suggests that there is indeed a limitation in seed arrival to such microhabitats (Verdú and García-Fayos, 1998). Our field observations indicated that *Daphne* seedlings are likely to recruit and become saplings underneath other nurse plants, as occurs in most plant species inhabiting in arid and semi-arid ecosystems (Callaway and Walker, 1997; Maestre et al., 2005). Interestingly, the genus *Daphne* belongs to the ancient Tertiary flora of the Mediterranean region which, according to the recent study by Valiente-Banuet et al. (2007), differs from species evolved during the increasingly dry Quaternary in species in that plant recruitment depends more strongly upon plant facilitation. Both *D. gnidium* (this study) and *D. rodriguezii* (Traveset and Riera, 2005; J. Rodríguez-Pérez and A. Traveset, unpubl. data) actually support the predictions of these authors.

Spatio-temporal discordance among recruitment stages

Discordance among stages in the recruitment process has been reported for a high number of Mediterranean species (Gulías et al., 2004; Lázaro et al., 2006; Rey and Alcántara, 2000; Traveset et al., 2003). We found that such discordance can be population dependent, as occurs in the olive grove in which there was coupling between recruitment stages. Thus, the highest seed rain (at least for one year) and seedling survival (both years) were consistently higher under olive trees, supporting the idea that this microhabitat had a facilitative effect on *Daphne* recruitment. The post-dispersal seed predation and seedling emergence stages, in contrast, were not altered by the microhabitat structure generated by seed dispersal and, consequently, they only act as a homogeneous (but fine) sieve along the recruitment process of *Daphne*. Directed dispersal by frugivores or seed arrival

to sites especially successful for survival (i.e., nurse plants) is, in fact, especially common in arid and semi-arid ecosystems (Wenny, 2001).

In the dune population, all seeds were deposited under *Daphne* and post-dispersal seed predation was consistently similar among microhabitats. However, both seedling emergence and growth were higher 'under *Pistacia*' which promoted discordance among the recruitment stages, despite seedling survival was independent of the shrub species identity of the microhabitat. Seed dispersal in the open was especially negative in this population, producing the lowest seedling emergence, survival and growth. In ecosystems in which water availability is crucial for plant recruitment, positive plant–plant interactions seem to prevail (Callaway et al., 1996; Ibáñez and Schupp, 2001) as a consequence of light reduction (Maestre et al., 2005), improvement of soil structure (Verdú and García-Fayos, 1996) and/or higher water content (Gómez-Aparicio et al., 2005). For the dune population, thus, recruitment patterns are mostly governed by processes acting during seedling stages.

So far, only a few studies have examined the effect of seed size on subsequent stages in plant recruitment (Alcántara and Rey, 2003; Gómez, 2004; A. Lázaro, unpublished data). In general, large seeds have shown to give a higher fitness in those stages mostly influenced by abiotic factors (e.g., seedling emergence, recruitment and growth). In our case, despite seed size positively affected seedling fitness in both *Daphne* populations, the effect was more important in the dune, which we attribute to the more stressful conditions under which germination occurs. A large seed, with a greater amount of resources in it, is more likely to survive a stressful environment. In the olive grove, the effect of seed size was independent of microhabitats but depended more on the specific climatic conditions of each year.

Heterogeneity in the recruitment stages across different scales

One of the central questions in plant demography is whether seedling recruitment can be predicted from seed rain (Jordano and Herrera, 1995; Schupp, 1995) or is more affected by other stages acting through the recruitment process (García et al., 2005; Kollmann, 2000). In a conceptual model developed by García et al. (2005), spatial concordance between seed dispersal and recruitment at a particular scale depends on the similarity in the spatial scales at which seed rain and post-dispersal losses operate. According to that model, concordance is expected when seed rain varies mostly at small scales while post-dispersal losses are most variable at a large scale, acting as a homogeneous filter for seed rain. In our system, we would predict that seedling

emergence is concordant with seed rain because post-dispersal seed predation losses do not differ at either the medium (microhabitat) or large (population) scales. The concordance between seed rain and seedling survival, however, varied between years and populations, occurring only in the olive grove in 2004; this population actually showed the highest abundance of adult plants under olive trees which might suggest that survival is consistently higher there than in the dune. The seedling survival stage mostly varies at small (site) and medium (microhabitat) scales, producing high variability in recruitment and, hence, discordance with seed rain is expected at a larger (population) scale (García et al., 2005).

Our findings stress the importance of distinguishing between critical stages (i.e., bottlenecks) in plant recruitment from the concordance between seed dispersal and post-dispersal stages. From a historical perspective, plant recruitment studies have mostly evaluated bottlenecks (e.g., Gulías et al., 2004; Lázaro et al., 2006; Rey and Alcántara, 2000; Traveset et al., 2003). However, there is growing evidence that the concordance (in terms of scale) between seed dispersal and post-dispersal stages is critical to predict plant recruitment patterns (García et al., 2005; Kollmann, 2000). In the present case, we have shown that both processes do not necessarily coincide within the same stage, even producing incongruent results. For example, there are stages, which represent a strong bottleneck in *Daphne* recruitment (e.g., post-dispersal seed predation) but promote low discordance with seed rain. By contrast, there are weak bottlenecks (e.g., seedling survival), which are highly discordant with seed rain, and consequently, are highly affecting the spatial structure of plant recruitment.

In short, the plant recruitment process of this *Daphne* species can be considered as a link of stages that are heterogeneous from a multiple-scale perspective. Such heterogeneity results from the different factors (e.g., frugivore's behaviour, rodent density, water availability), the effect of which is not necessarily consistent through the recruitment process, influencing each specific stage.

Acknowledgements

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