Transition probabilities from pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-salvatoris*) in two habitats

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Summary

1 We elucidated the critical life stages and processes in the recruitment of *Rhamnus ludovici-salvatoris*, a dioecious shrub endemic to the Balearic Islands (western Mediterranean), populations of which have been shrinking in recent decades. We evaluated transition probabilities between all stages involved in regeneration from pollination to seedling recruitment, in order to determine the overall probability of an ovule in a flower becoming an established individual in a population.

2 We compared populations in an oak (*Quercus ilex*) forest and in a more disturbed habitat (an abandoned field). We studied seed rain and probabilities of predation, germination and survival in the main microhabitats within each community.

3 High levels of seed predation after dispersal and unfavourable conditions (water deficit) during germination and recruitment periods were the most critical factors limiting population growth in both habitats.

4 The greater fecundity of shrubs in the abandoned field did not compensate for the lower probability of ovules recruiting new individuals in that habitat compared to the forest.

5 Spatial patterns of recruitment in this species cannot be predicted from seed dispersal patterns produced by frugivorous birds. In both habitats, but especially in the forest, there was strong spatial discordance between seed rain (most seeds were deposited under conspecifics) and seedling establishment. In the forest, most established *Rhamnus* are found under oaks, whereas in the abandoned field, saplings and juveniles are more equally distributed across different microhabitats.

6 This empirical study supports the idea that habitat disturbance, in general, may considerably alter the patterns of plant recruitment. For endemic species with restricted and rather small populations, the loss of natural habitat is likely to be detrimental for species' persistence, as recruitment in disturbed sites is greatly reduced.

Key-words: Balearic Islands, Mediterranean habitats, population recruitment, seed dispersal by birds, seed and seedling survival, spatial variation, disturbance

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Introduction

The consequences of pollination success and seed dispersal for plant populations can only be fully assessed by evaluating the consecutive losses of potential new individuals at different stages in the reproductive cycle. Even though ecologists have been conscious of this for

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a long time (Harper 1977; Louda 1982; Dirzo & Domínguez 1986; Howe 1989), it was not until the last decade that studies bridging those different stages were carried out. The process of seed dispersal has often been linked to that of seedling recruitment (e.g. Houle 1992, 1998; Herrera *et al*. 1994; Clark *et al*. 1999; Nathan *et al*. 2000a,b; Holl 2002), but few studies have also considered the previous stage, i.e. that of pollination (but see Herrera 2000; Picó & Retana 2000). Such an integrative approach requires much effort as it

requires simultaneous monitoring of flower and fruit production, seed dispersal, seed losses to predators, seed rain in different microhabitats, germination patterns and seedling survival rates. However, only with such an approach can the critical steps that determine individual fitness be assessed. Furthermore, the spatial and temporal patterns of variation in fitness between individual plants need to be considered if our goal is to understand the demography and population dynamics of a particular species.

In the case of vertebrate-dispersed plants, on which most 'linking-stages' studies have been carried out, frugivores may limit seedling recruitment by depositing many seeds in sites that are unsuitable for germination and future growth. On the other hand, seed dispersers may influence the spatial distribution of seedlings by disseminating different amounts of seed in an array of microhabitats. Data reported so far show little evidence of spatial concordance ('coupling') between seed dispersal and subsequent processes, as expected from the usual interdependence of factors that affect each stage of the life cycle (e.g. Jordano & Herrera 1995; Schupp 1995; Rey & Alcántara 2000).

The objective of our study was to determine the crucial life stages in recruitment of *Rhamnus ludovici-salvatoris* R. Chodat, a shrub endemic to the Balearic Islands, that has suffered reductions in size and number of populations in recent decades. This reduction is mainly due to deforestation but also probably to climatic change that this Mediterranean region has experienced during the Holocene (Yll *et al.* 1997). We quantified the processes affecting each stage of regeneration and calculated transition probabilities between stages in two habitats; one a relatively undisturbed oak forest and the other an abandoned almond and olive field that had been cultivated in the past. As far as we know, this is the first time that spatial variation in transition probabilities has been examined from pollination to recruitment. Our specific questions were as follows. (1) Is seed production limited by pollination or by any factor affecting seed viability? (2) Is seed dispersal limited by avian fruit removal? (3) How important are post-dispersal predators, especially vertebrates (rodents) and ants, in limiting recruitment? (4) Is there strong spatial discordance between the different stages from seed rain to recruitment? (5) Do the critical life stages differ between the two habitats, and if so, how?

Materials and methods

STUDY SPECIES

Rhamnus ludovici-salvatoris (Rhamnaceae) is a perennial shrub up to 2 m tall, endemic to the Gymnesic Islands (Mallorca, Menorca and Cabrera) of the Balearic Archipelago. In Mallorca, where most populations are located, it occurs from sea level up to 1200 m a.s.l., usually in oak (*Quercus ilex*) forests, and more rarely in dry garrigues. Although it is a dioecious species,

males may eventually produce a few female flowers, and vice versa. It flowers during spring, with a peak at the end of April to mid-May. Inflorescences are fascicles, bearing 7–8 pale yellow flowers; these produce nectar which is collected by bees, dipterans, small beetles and ants. Full-sized fruits occur about two months after anthesis; they are black when mature and contain an average of two seeds (*n* = 500). Fruits are consumed mostly by two frugivorous birds, *Turdus merula* and *Sylvia melanocephala*, which digest the pulp but pass the seeds intact; these are the only two avian frugivores present in the area during summer. As in other *Rhamnus* species, seeds bear an elaiosome and may thus be secondarily dispersed by ants (Bas 2001). *Crematogaster scutellaris*, *C. laestrygon* and *Pheidole pallidula* are the species found in our study areas; the two former are usually inefficient dispersers whereas the latter may occasionally disperse seeds secondarily (Bas 2001). The mouse *Apodemus sylvaticus* preys upon the seeds of *R. ludovici-salvatoris*, mainly in the forest site. This shrub produces a negligible seed bank, as is the case with other congeneric species (Bas 2001).

STUDY SITES

The study was performed in two sites that differed mainly in the degree of disturbance, but also in the density of *R. ludovici-salvatoris* (hereafter *Rhamnus*) shrubs, type of vegetation, elevation and climatic conditions. The oak forest site is located at *c.* 600 m elevation, near the Lluc Monastery (39°49′ N, 2°53′ E) (hereafter Lluc). The density of *Rhamnus* is about 100 individuals ha^{-1}; we located 41 males and 37 females, a ratio that does not differ significantly from 1 : 1 (χ^2 = 0.21, *P* > 0.05). Mean annual precipitation is 1265 mm (mean for 20 years; Guijarro 1986); mean maximum temperature of the warmest month (August) is 27.5 °C and mean minimum temperature of the coldest (January) is 2.5 °C. The vegetation cover at this site is mainly *Quercus* (47%), *Pistacia lentiscus* (21%), *Cistus monspeliensis* (10%), *Pinus halepensis* (9%), *Cneorum tricoccon* (5%) and *Rhamnus* (3%). The second population is located at 140 m elevation, *c.* 5 km from Estellencs (39°39′ N, 2°27′ E). Mean annual precipitation is 713 mm, and mean maximum and minimum temperatures are 27.0 °C and 4.0 °C, respectively (Guijarro 1986). This site (hereafter Estellencs) is an abandoned field, in which olive (*O. europaea*) and almond (*Prunus dulcis*) trees are the most abundant species, accounting for 31% and 27%, respectively, of the total vegetation cover. Other common species in the area are *Smilax aspera* (17%), *Ampelodesma mauritanica* (13%), *Chamaerops humilis* (6%) and *Rhamnus* (4%). Density of *Rhamnus* shrubs in this population is lower (*c.* 60 individuals ha^{-1}) than at Lluc, and sex ratio is also 1 : 1 here (χ^2 = 2.86, *P* > 0.05) (a total of 36 males and 23 females were recorded within approximately a hectare). No spatial segregation of sexes was found at either site.

The size of *Rhamnus* shrubs is significantly greater at Estellencs than at Lluc (mean height: 152.5 ± 13.5 cm vs. 93.9 ± 12.1 cm; crown diameter: 159.0 ± 13.5 cm vs. 90.0 ± 10.1; $n = 20$ and $n = 17$, respectively). There were no differences in height or diameter between sexes at either site. Fruit crop is also much greater at Estellencs (*c.* 1998 ± 824 ($n = 10$) vs. 245 ± 68 ($n = 7$) fruits per female plant; data from 1999). Fruits, in contrast, are somewhat larger at Lluc (76.4 ± 1.5 (SE) mg in weight; $n = 287$) than at Estellencs (56.9 ± 0.6 mg; $n = 210$), having more pulp, probably due to the higher water availability at the former site. Seed weight is similar in the two populations (*c.* 4 mg; $n = 273$). The distance from the monitored females to the nearest reproductive male was similar in both populations (2.05 ± 0.35 m; $n = 10$ vs. 2.35 ± 0.89 m; $n = 7$).

FLOWERING PHENOLOGY

In the spring of 1999 we monitored flowers on a minimum of three labelled branches per plant, from 10 males and 10 females at each site, throughout the entire flowering period (from 8 April to 5 May at Estellencs, and from 21 April to 17 May at Lluc). Every week we recorded the number of buds, open flowers and withered flowers. For each female plant, we estimated (following Augspurger 1983) an index of flowering synchrony, which varies from 0 (no overlap between sexes) to 1 (total overlap).

SEXUAL SYSTEM

The sexual system of *Rhamnus* was first examined at Lluc in spring of 1998. On 2 April, we counted and bagged buds from 10 female plants to (i) assess whether pollen limits fruit set (from 15–21 April we hand-pollinated flowers at anthesis with pollen from different males), and (ii) determine the potential role of wind as a pollinating vector (the bags allowed pollen to pass through but not insects). Another group of unmanipulated (open-pollinated) flowers served as controls. A minimum of three branches, with similar numbers of flowers, was used for each treatment and control. Fruits produced were recorded on 31 May. In April 1999, we repeated the pollination experiments on this population with the same individuals (except for three females that did not flower), and performed them also on 10 female plants at Estellencs. Fruits were counted on 18 May and 3 June, respectively, at the two sites. The possibility of apomixis was examined in 1999 at Estellencs by bagging female buds and not allowing the passage of either pollen or insects; these bags were removed after flowers had withered and the stigma was no longer receptive.

SEED DISPERSAL AND SEED RAIN

Both rate of fruit removal and seed rain were studied in the two populations during summer 1999. Branches from each female shrub used in the study of the sexual system were periodically (every 7–10 days) monitored for fruit removal until fruits had dried or were dropped. Natural fruit dropping was monitored on bagged branches. Seed rain was evaluated in a total of five microhabitats at Lluc and four at Estellencs: (i) open spaces, with no vegetation cover, (ii) under female conspecifics and (iii–v) under the most abundant tree or shrub species, i.e. those most likely to act as perches for birds in each habitat; these were *Quercus*, *Pistacia* and *Pinus* at Lluc, and *Olea* and *Prunus* at Estellencs. Both seeds and fruits were collected in aluminium trays (33 cm long, 26 cm wide and 4.5 cm deep) covered with wire mesh to avoid predation by vertebrates. Ten replicates per microhabitat and two trays per replicate were used. Each sampling station (i.e. a pair of trays) was randomly placed throughout the area so that distance from each sampling station to any reproductive female *Rhamnus* was similar between microhabitats. Monitoring extended throughout the ripening period, from 30 June to 1 September at Lluc, and from 25 June to 10 August at Estellencs. During this period, we visited each site three times, recording number of entire fruits and total number of endocarps (each endocarp corresponding to a single seed) for each tray. We recorded endocarps rather than seeds because some seeds had already been detached from their endocarps and had been lost due either to ant removal or to seeds having bounced out of the tray when the endocarp dehisced.

FRUIT PREDATION

The level of fruit predation was studied during the same period and in the same microhabitats and populations as in the seed rain experiment. Two treatments were applied, one in which fruits were available to all predators (control) and one in which vertebrate predation was prevented by means of a 1-cm wire mesh. Five replicates were used per microhabitat and treatment, each with five ripe fruits placed in a Petri dish. Each pair of dishes (covered and uncovered) was arranged near the sampling stations used for the seed rain experiment. Both populations were visited twice, and the number of removed fruits was recorded at each visit.

SEEDLING EMERGENCE AND SEEDLING SURVIVAL

Rhamnus seeds were sown on 16 and 18 November 1999 at Estellencs and Lluc. Three microhabitats were used: open (no vegetation cover), under female conspecifics and under the tree species where seed rain had been highest: *Quercus* at Lluc and *Olea* at Estellencs. Each sowing unit consisted of 24 seeds, in a matrix of four rows and six columns, separated by 5 cm, and planted at a depth of 0.5–1 cm. Each replicate was protected with a cage (30 × 20 × 2.5 cm) made of wire

mesh to prevent rodent predation. Five replicates were used per microhabitat. We removed all naturally occurring seeds from these sites before the experiment began. Litter was added, to match the natural conditions as closely as possible. Seedling emergence and mortality were monitored every 2–3 weeks during an entire year. In the fall of 2000, we repeated the sowing experiment at the same sites as in 1999 due to the very low number of seedlings emerged. We planted the same number of seeds on 2–3 November and monitored seedling emergence until autumn 2001. Most seeds germinated in winter, although they still bore the cotyledons in April 2001.

In addition to our sowing experiments, we recorded seedling emergence in the spring of 2002 in the two populations, as this year was especially rainy during this season compared to the previous 3 years (rainfall from January to April 2002 was more than twice the average of the period 1999–2001). We searched intensively for seedlings in the two localities, using 50×2 m transects – with a separation of *c.* 5 m between them – that were more or less homogeneous and that covered the entire area where *Rhamnus* is found. We examined a total of 600 m^2 and 700 m^2 at Estellencs and Lluc, respectively, labelling all seedlings (all had fewer than five leaves, and usually still bore the cotyledons) and recording for each one: length, number of leaves and microhabitat. At the end of October 2002, we recorded survival of those seedlings to determine the proportion that survived the harsh summer conditions. To find out how recruitment sites matched germination sites, we further recorded and measured saplings and juveniles (approximately less than 5 years old) on those transects, along with the microhabitat in which they had established.

Seed germination was also tested in the laboratory under controlled conditions (12 h of total darkness at 10 °C, 12 h of light at 20 °C). A total of 200 seeds from a minimum of 10 individuals from each population, with the endocarp removed, were placed in Petri dishes (25 seeds per dish; 8 dishes). All seeds that did not germinate within 3 months were dissected to determine the proportion that was non-viable.

TRANSITION PROBABILITIES (TPS)

We calculated a total of six transition probabilities between different stages from pollination to seedling survival, the first two of which were calculated on a per tree basis, and the other four on a microhabitat basis:

1. TP$_1$ was equivalent to fruit set, i.e. number of mature fruits relative to number of flowers (fruit set may not be equivalent to 'pollination success' *per se*, as fertilized ovules may have aborted before reaching maturity). In *Rhamnus*, fruit set is almost equivalent to seed set (seeds/ovules) as flowers bear two ovules and a fruit usually contains two seeds.

2. TP$_2$, the probability that a fruit was removed by a bird (regardless of seed fate).

3. TP$_3$, the probability that a seed was deposited in a particular microhabitat (estimated as mean density of seeds dispersed in each microhabitat relative to the sum of mean seed densities deposited in all microhabitats considered).

4. TP$_4$, the probability of escaping predation (by either rodents or ants) in each microhabitat.

5. TP$_5$, the number of seedlings emerging relative to the number of viable seeds planted in each microhabitat (i.e. the proportion of seedlings emerged times the proportion of viable seeds; seed viability tested in the lab).

6. TP$_6$, the probability of seedling survival (which would allow a sapling and then a juvenile to establish) in each microhabitat. The comparison of this probability between microhabitats could be done only in 2002, when a sufficiently large sample size of seedlings was obtained. We evaluated the probability that a seedling could survive the summer (the most stressful season).

When interpreting these TPs, we need to consider that TP$_4$ and TP$_5$ were calculated assuming that the same number of 'units' entered each new stage in each microhabitat, as (i) seed rain in some microhabitats was zero and (ii) seed predation was intense in most of them. TP4 is also likely to be over-estimated, as fruits or seeds may be easier for rodents or ants to detect on Petri dishes than when naturally buried by litter (Hulme 1998). TP$_5$ and TP$_6$ could not be estimated from the cohort of seeds produced in 1999 due to negligible germination, and thus were calculated from data gathered the next year, when germination was slightly greater. Other estimates of TP$_5$ and TP$_6$ were obtained from data collected in 2002, when much greater germination occurred in both populations.

In order to estimate the overall probability of recruitment (OPR) in the population, it was necessary to weigh the cumulative probability of recruitment (CP, which is the product of all TPs) by the relative abundance of each microhabitat in the area. We multiplied the CP in each microhabitat by the proportion of area covered by that microhabitat, and OPR was thus the sum of all those products.

Data analysis

Most statistical analyses performed were parametric (ANOVAs and correlations). Angular transformations were used to normalize the proportions in different tests. When data could not be normalized, non-parametric tests (Spearman correlations and Mann–Whitney *U*-test) were employed. Differences between microhabitats in the frequency of seedlings and juveniles were tested by means of Chi-square tests whereas differences in seedling survival were analysed with Generalized Linear Models ANOVA, using binomial error distributions and logit link functions. Microhabitat was a fixed factor and both seedling length and number of leaves per seedling were used as continuous covariates. The statistical package employed was Statistica 5.5 (StatSoft Inc. 1999).
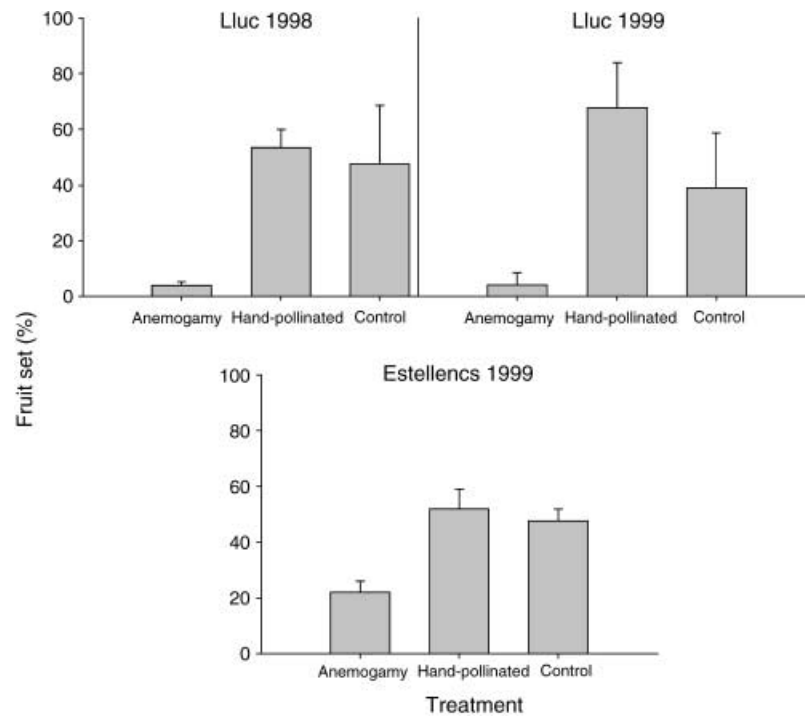
Fig. 1 Proportion of flowers from the different pollination treatments that set fruit in the two populations of *R. ludovici-salvatoris* in the two habitats. Bars on columns represent one standard error.

Results

THE PROCESS OF POLLINATION

The two sexes coincided in their flowering peak in both populations (mid and late April for Estellencs and Lluc, respectively). Flowering synchrony index was 0.91 and 0.96, respectively, indicating that pollen was available throughout almost the entire period when stigmas were receptive. The species was not apomictic. Figure 1 shows the proportion of flowers that produced mature fruits for each treatment. Wind was responsible for only 4% of the fruit set in the two study years at Lluc; in contrast, anemogamy at Estellencs resulted in 22% of fruit set, probably because this coastal population is more exposed to winds. At Lluc, greater fruit set in hand-pollinated flowers compared to controls ($F_{2,18} = 33.21$, $P < 0.001$) indicated that there was some pollen limitation, although this was observed only in 1999 (Fig. 1).

Fruit set was not correlated either with variables describing plant size, flowering synchrony with males in the population, or with distance to the nearest reproductive male, although more fecund females tended to set greater proportions of fruits ($r = 0.82$, $P = 0.02$), at least at Lluc.

Viability tests in the laboratory showed that most seeds in mature fruits were viable in both populations (92% and 85%, at Lluc and Estellencs, respectively). No pre-dispersal seed predation (by insects or fungi) was detected in either population, and thus we did not consider it in the calculation of transition probabilities.

THE SEED DISPERSAL PHASE

The production of fruits at Estellencs was, on average, eight times greater than at Lluc. Fruit removal was intense (over 95% of the fruits had disappeared within 1 month) at both sites. Seed rain differed significantly between microhabitats (Table 1), being greatest under female conspecifics at both sites. Most fruits under females had fallen directly from the plant, but others had been dropped by birds, as shown by beak marks. At Estellencs, the second highest seed density was under *Olea*. Lowest densities were found under *Prunus* and in the open. In contrast, at Lluc, the second highest seed density was in the open, followed by *Quercus*. Very few seeds were found under *Pinus* and none under *Pistacia*.

The probability of seeds escaping post-dispersal predation was very low in all microhabitats in both populations (Table 1). At Estellencs, 12 days after placing the fruits in the dishes, removal was over 50% both in the vertebrate-excluded and open treatment, and nearly 100% 2 weeks later (Fig. 2a). In this site, removal was mainly due to ants, presumably *Cremato-gaster scutellaris* and *Pheidole pallidula*, which are common in the area. No significant differences between microhabitats were found in this population, and there was no significant interaction between the effects of microhabitat and vertebrate-exclosure (presence/absence) (all $P > 0.05$). In contrast, at Lluc, fruit/seed removal by vertebrates (presumably *Apodemus sylvaticus*, which is known to be in this forest area) was much more important than at Estellencs (Fig. 2b). Removal was almost 100% in the open dishes whereas it was significantly lower in the vertebrate-excluded

Table 1 Seed rain and seed predation in different microhabitats. Due to the large number of empty trays, figures in the seed density column are the summed densities in all replicates (*n* = 10) for each microhabitat. For predation, values represent mean ± 1 SE

Population	Microhabitat	Seed density*	Seed predation† (%)
Estellencs	*Rhamnus*	702.33 (469.77)	100 ± 0
	Olea	4.07	100 ± 0
	Prunus	1.74	100 ± 0
	Open	1.16 (0.58)	100 ± 0
Lluc	*Rhamnus*	8.72 (36.63)	100 ± 0
	Pistacia	0	100 ± 0
	Quercus	1.16	80 ± 20
	Pinus	0.58	100 ± 0
	Open	1.74	92 ± 8

*The number in parenthesis indicates the density of entire fruits found in this microhabitat.
†By both rodents and ants, *n* = 5 replicates per microhabitat.
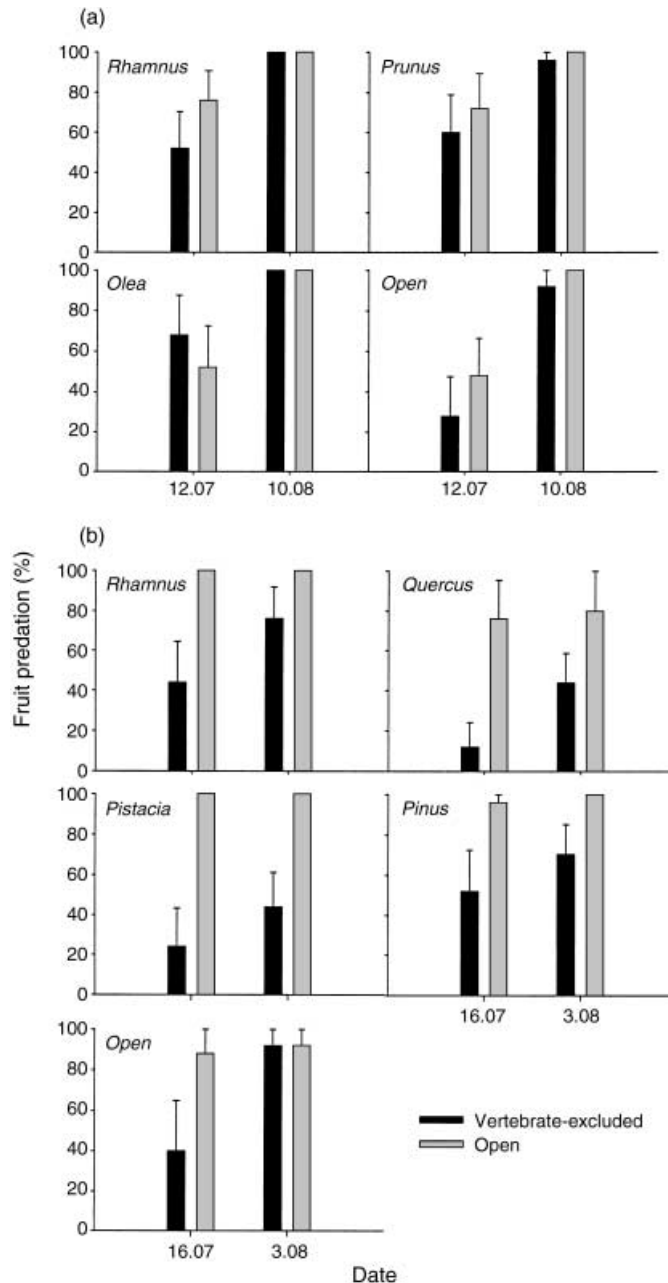
Fig. 2 Cumulative post-dispersal seed predation in the different microhabitats of (a) the abandoned-field population and of (b) the forest population. Bars on columns represent one standard error. Data from 1999.
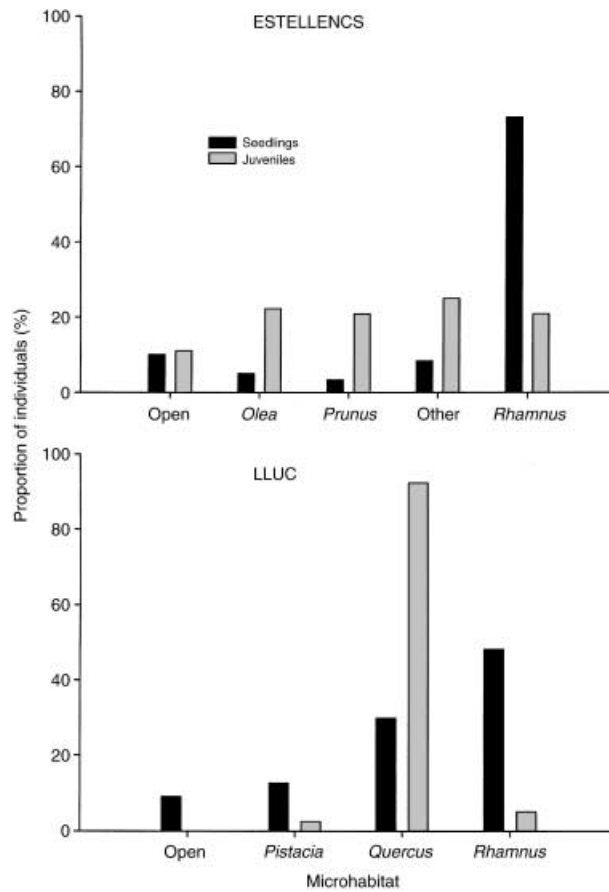
Fig. 3 Proportion of seedlings and juveniles found in the different microsites in each population. Data from 2002. At Estellencs, 'Other' represents other vegetation (mainly *Calicotome* and *Ampelodesma*).

ones ($F_{1,40} = 32.94$, $P < 0.001$); such differences were consistent between microhabitats ($F_{4,40} = 0.36$, $P > 0.05$) and no significant interaction was found ($F_{4,40} = 1.27$, $P > 0.05$).
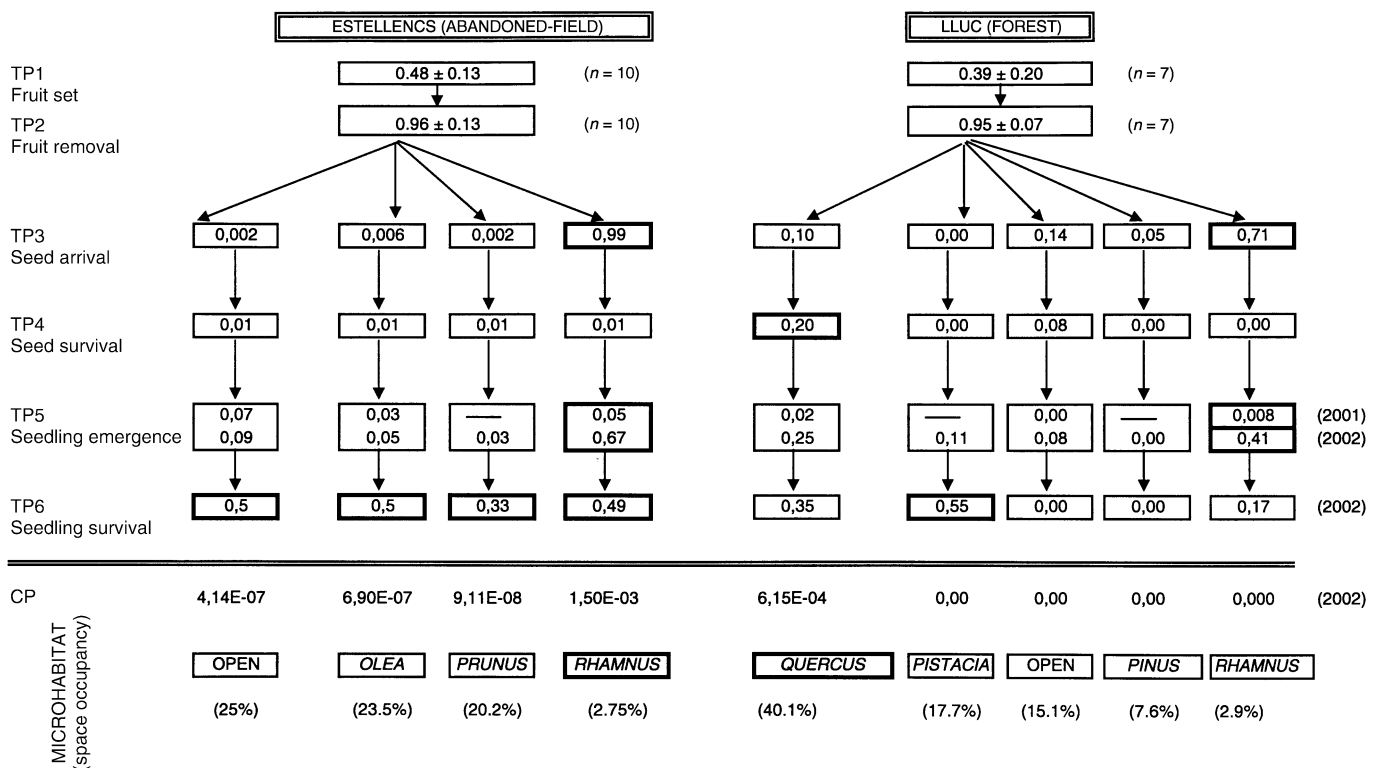
THE SEEDLING STAGE

From the seeds planted in autumn 1999, only six seedlings (0.8%) emerged, two at Estellencs (one under *Rhamnus* and one in the open) and four at Lluc (three under oak trees and the other under *Rhamnus*). In the laboratory, in contrast, germination was 72% for seeds from Estellencs and 47% for seeds from Lluc, suggesting that the low germination in the field may have been due to the extreme dryness during this year. We do not think that sown seeds were preyed upon by ants that entered the vertebrate-exclusion cages as burial usually protects seeds from these insects (Hulme 1998). Seeds sown in 2000 had slightly higher germination. At Estellencs, germination was similar in all study microhabitats, but only 5% of the planted seeds germinated (19 seedlings). At Lluc, only one seed germinated out of the 360 seeds sown. All seedlings died during the summer.

The importance of precipitation for germination was confirmed in 2002. This was the first year that we observed abundant seedlings in the field. At Estellencs

we recorded a total of 119 seedlings on the transects. As shown in Fig. 3, most were located underneath *Rhamnus* shrubs (65.5% under females and 34.5% under males). At Lluc, we recorded a total of 87 seedlings, almost half under *Rhamnus* (88% were under females and the rest under males) and a large proportion under oak trees (Fig. 3).

At the end of October 2002, seedling survival at Estellencs was *c.* 50%, and it did not differ significantly between microhabitats ($\chi^2 = 1.23$, $P = 0.87$, d.f. = 4) (Fig. 4). Survival appeared to be influenced by the number of leaves a seedling bore in spring ($\chi^2 = 4.77$, $P = 0.03$, d.f. = 1), although not by its length ($\chi^2 = 0.05$, $P = 0.83$, d.f. = 1). A separate logistic regression considering only number of leaves per seedling in the model confirmed a significant effect of this variable on survival ($\chi^2 = 4.55$, $P = 0.03$, d.f. = 1). At Lluc, seedling survival was half that at Estellencs, and it differed significantly between microhabitats ($\chi^2 = 13.35$, $P = 0.004$, d.f. = 3) (Fig. 4). Seedling size here did not affect the probability of survival ($\chi^2 = 2.93$, $P = 0.09$, d.f. = 1, and $\chi^2 = 2.50$, $P = 0.11$, d.f. = 1, for seedling length and number of leaves, respectively).

The patterns of seedling survival in the two populations are consistent with the spatial distribution of saplings and juveniles (due to the low number of the former both categories were pooled; Fig. 3). At

	ESTELLENCS (ABANDONED-FIELD)				LLUC (FOREST)				
TP1 Fruit set	0.48 ± 0.13 (n = 10)				0.39 ± 0.20 (n = 7)				
TP2 Fruit removal	0.96 ± 0.13 (n = 10)				0.95 ± 0.07 (n = 7)				
TP3 Seed arrival	0,002	0,006	0,002	0,99	0,10	0,00	0,14	0,05	0,71
TP4 Seed survival	0,01	0,01	0,01	0,01	0,20	0,00	0,08	0,00	0,00
TP5 Seedling emergence (2001)	0,07	0,03	—	0,05	0,02	—	0,00	—	0,008
TP5 Seedling emergence (2002)	0,09	0,05	0,03	0,67	0,25	0,11	0,08	0,00	0,41
TP6 Seedling survival (2002)	0,5	0,5	0,33	0,49	0,35	0,55	0,00	0,00	0,17
CP (2002)	4,14E-07	6,90E-07	9,11E-08	1,50E-03	6,15E-04	0,00	0,00	0,00	0,000
MICROHABITAT (space occupancy)	OPEN	OLEA	PRUNUS	RHAMNUS	QUERCUS	PISTACIA	OPEN	PINUS	RHAMNUS
	(25%)	(23.5%)	(20.2%)	(2.75%)	(40.1%)	(17.7%)	(15.1%)	(7.6%)	(2.9%)

Fig. 4 Diagram of the spatial dynamics of recruitment of *R. ludovici-salvatoris*, showing the transition probabilities (TP) at each stage and the cumulative probability (CP) for all microhabitats. Thicker lines around each box reflect greater suitability of that microhabitat compared with other microhabitats for recruitment within each stage. Diagram based on fig. 2 in Rey & Alcántara (2000).

Estellencs, the proportion of juveniles was similar in all microhabitats ($\chi^2 = 3.94$, $P = 0.41$, d.f. = 4). In contrast, significant differences were found at Lluc ($\chi^2 = 86.32$, $P = 0.001$, d.f. = 3), where 92% of juveniles were located under oak trees (Fig. 3).

TRANSITION PROBABILITIES BETWEEN STAGES AND OVERALL PROBABILITY OF RECRUITMENT

Figure 4 summarizes the transition probabilities between all stages considered in this study. Potential recruits were reduced by *c.* 50% at Estellencs and *c.* 60% at Lluc before the dispersal phase due to the rather low proportion of flowers that set fruit. However, post-dispersal seed predation and seedling recruitment (specifically, seedling survival within the first year) were the most critical stages. Seedlings are usually submitted to a rather strong water stress during the summer and, as shown by the data from 2002, seedling mortality in this period was very high in both populations. For years with little rain, such as 1999–2001, germination also represents a severe bottleneck in the *Rhamnus* life cycle.

At both sites, transition probabilities between stages differed considerably between microhabitats, i.e. there was no spatial concordance in different phases of the life cycle. A particular microhabitat did not consistently provide the highest probability of survival to the next stage (Fig. 4). The CPs (i.e. the product of all TPs in a microhabitat) showed that the highest probability of an ovule of a flower reaching the seedling stage and surviving, at least over the summer, occurred in different microhabitats in the two populations (under conspecific adults at Estellencs and under oaks at Lluc). When considering the relative abundance of each microhabitat, OPR at Estellencs proved to be about six times lower than at Lluc, mainly because the favourable *Rhamnus* microhabitat occupies less than 3% of the area in contrast to the 40% occupied by oaks at Lluc. Our estimates of OPR indicated that an ovule at Estellencs had a probability of 0.005% of becoming a seedling and surviving the summer, whereas at Lluc this probability was *c.* 0.03%.

Discussion

FACTORS INFLUENCING THE LIFE-CYCLE AND RECRUITMENT DYNAMICS OF *RHAMNUS LUDOVICI-SALVATORIS*

The production of seeds appeared to be somewhat limited by pollen availability, although this was found to vary spatially and temporally, as in other Mediterranean species (especially in early flowering plants; Baker *et al.* 2000; references therein). In insect-pollinated flowers, fruit set is likely to depend upon insect densities and activities which are known to vary spatially

and temporally due to a variety of biotic and abiotic factors (Stiling 1988). We found pollen to be limiting only in one of the years in the forest population. In this population anemogamy plays a minor role in pollination (< 5% of fruit set during 2 years) and seed production is more dependent upon insects. Also, in this site only, larger fruit crops had higher fruit sets, suggesting that small plants (with usually small crops) may attract fewer insect pollinators. The greater fruit set found in the abandoned-field (*c.* 50%) compared to the forest site (*c.* 40%) might be due, at least partly, to the more important role in pollination played by wind at this site (22%). On the other hand, the larger size of *Rhamnus* shrubs at Estellencs might result in higher fruit set simply because of greater resource availability.

A lack of resources (mainly water) probably contributed to the decrease in the potential seed production in our study cohort. Seed viability was high in 1999, although this also varied spatially and temporally; data obtained in 2000 showed 42% and 26% seed abortion at Estellencs and Lluc, respectively. The levels of seed abortion may depend upon different factors, e.g. temperature, rainfall and nutrient availability. The possibility of some inbreeding depression in the abandoned-field population also exists, as the number of individuals is much lower than in the forest population.

Fruit removal by birds was very high at both sites. This is presumably due to the scarcity of other fleshy fruits in early mid summer and to the abundance of sedentary frugivorous birds such as blackbirds and Sardinian warblers. In both populations, seed rain was highest under conspecifics (mainly beneath females), especially in the abandoned-field. Maximal seed rain under conspecific plants has been reported for other fleshy-fruited bird-dispersed species (Alcántara *et al.* 2000; references therein; Holl 2002) and is attributed to (i) birds spending long periods of time perching on the plants from which they feed and (ii) the dropping of fruits that remain undispersed.

Post-dispersal seed predation, mainly by ants in the abandoned-field and by rodents in the forest site, caused considerable seed loss in all microhabitats and was found to be a critical step in the life cycle of *Rhamnus* in both habitats. Some secondary seed dispersal by ants is not discarded, although *Crematogaster* and *Pheidole* are not efficient dispersers for this or other plant species (Bas 2001; Passos & Oliveira 2002). The number of seed fragments found in the dishes in fact indicated that most seeds were preyed upon, and that secondary dispersal was minimal. High seed mortality due to post-dispersal seed predators has been reported for a number of species from the tropics (e.g. Schupp 1990, and references therein; Wenny 2000) and from the temperate zone (e.g. Traveset 1994; Hulme 1997). Whether this consistently limits seedling recruitment every year depends on ant and rodent population dynamics in each area. Such populations may fluctuate dramatically from year to year and shift spatial patterns of attack in response to a variety of factors (tem-

perature, humidity, predator abundance, etc., e.g. Houle 1992; García *et al.* 2001).

The long period of water stress suffered by plants from 1999 to 2001 had a strong impact on the phases of seed germination and seedling recruitment, and caused mortality of all the seedlings that emerged in both habitats. The importance of drought was confirmed in 2002, when rainfall was much more abundant than in the three previous years and large numbers of seedlings were observed, of both *Rhamnus* and other coexisting species. Summer drought is the main cause of seedling mortality and represents a bottleneck in the life cycle of other Mediterranean species such as *Helianthemum squamatum* (Escudero *et al.* 1999), *Olea europaea* (Rey & Alcántara 2000) and *Ampelodesmus mauritanica* (Vilà & Lloret 2000). The probability of a seedling surviving the summer, at least in the abandoned field, was correlated with the number of leaves it had before this season, which suggests that a greater number of leaves might mean lower stress for a seedling.

SPATIAL DISCORDANCE BETWEEN STAGES AND DIFFERENCES BETWEEN HABITATS

In the abandoned field habitat, the spatial pattern of seed rain across microhabitats was concordant with that of seedling emergence, which indicates that the pattern of seed mortality due to biotic and abiotic factors does not modify that generated by avian seed dispersers. The similar levels of post-dispersal seed predation in all microhabitats certainly contribute to such coupling between seed rain and seedling emergence stages. Seedling emergence and sapling recruitment, however, were discordant. Although the probability of surviving the summer did not differ between microhabitats, the spatial distribution of saplings and juveniles indicates that larger proportions of seedlings die under conspecifics (probably due to intraspecific competition, herbivory, pathogens, etc.) than in the other microhabitats. In contrast to the abandoned field, spatial discordance was found in the forest site between seed rain and seedling emergence patterns. The microhabitat under *Rhamnus* had the highest probability of seed arrival, but seed survival was highest under *Quercus*. There was also spatial discordance between seedling emergence and recruitment of saplings and juveniles. Although most seedlings emerge under conspecifics, the large majority of recruits are under oaks.

Spatial discordance between stages has been reported in a number of studies (e.g. Houle 1992, 1998; Herrera *et al.* 1994; Rey & Alcántara 2000; Vilà & Lloret 2000; see review in Clark *et al.* 1999), and has been attributed to biotic (e.g. herbivores, competitors and pathogens) as well as abiotic (e.g. temperature, radiation, relative humidity and soil nutrients) factors. Temporal variation in the influence of such factors will determine whether a given microhabitat consistently has the greatest probability of recruitment (e.g. Schupp

& Fuentes 1995; Houle 1998; Ibáñez & Schupp 2001). Seed rain patterns have been found to be consistent in time in some species (Schupp 1995; references therein). For *Rhamnus*, the patterns of seed rain are also likely to be rather constant from year to year, as few bird species feed upon the fruits of this plant in either habitat and they probably have similar foraging and perching habitats from one year to the next (no other bird-dispersed plants bear fruits at the same time). Post-dispersal seed predation patterns, in contrast, are likely to vary from season to season, mainly depending upon abundances and activities of seed predators. To assess this possibility in our case would obviously require a longer study.

Recruitment in plant populations is usually affected by a combination of factors such as seed limitation and availability of microsites, which in turn may be determined by seed production, dispersal, seed mortality due to different causes, frequency of disturbances and also by the environmental conditions prevailing at the time of seedling emergence and establishment (Kiviniemi & Eriksson 1999). For *Rhamnus*, seed limitation appears to be more important than availability of microsites in both habitats. In the abandoned field, there is seed rain limitation due to the activity of avian frugivores; the large majority of seeds fall under the conspecific plant and only a small fraction are dropped in microhabitats that appear to be suitable for establishment. In addition, high levels of post-dispersal seed predation and water stress during germination and recruitment appear to limit population growth. In the forest, a larger fraction of seeds is dropped by birds in microhabitats other than under conspecifics than in the abandoned field. Although seed predation and water stress also strongly affect germination and establishment, the greatest probability of seed and seedling survival under the most abundant microsite (below oaks) results in less population growth limitation at this site.

In conclusion, the differences observed between the two habitats demonstrate that patterns of plant recruitment can vary notably at a spatial scale, and that population growth limitation can be greater in disturbed sites. In a rare endemic species such as *Rhamnus ludovici-salvatoris*, the effects of a disturbance such as the loss of natural habitat are even more important, as they can lead to the extinction of some of its decreasing populations.

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