Seed dispersal effectiveness in a plant–lizard interaction and its consequences for plant regeneration after disperser loss

Javier Rodríguez-Pérez · Anna Traveset

Abstract Mutualistic disruptions, such as those promoted by the loss of seed dispersers, can have negative effects on the plant regeneration of those species that strongly depend upon them. In order to adequately assess how plant communities are affected by such disruptions, we need to know the importance of the dispersal phase, both in its quantitative and qualitative components. We examined this in the narrow interaction between the shrub *Daphne rodriguezii* and its (only) disperser, the lizard *Podarcis lilfordi*. We quantified fruit removal and the effect of fruit/seed-size selection, seed treatment in the disperser's guts and seed deposition patterns on seedling emergence and survival. In the only locality in which lizards persist, they removed most fruits and showed preference for larger ones in one of the two study years. Seed treatment in lizard's guts had no effect on germination, although it tended to reduce the effect of seed size on germination (differences between large vs. small seeds in seed germination were higher for non-ingested seeds). Probability of seedling emergence, but not survival, was higher in the locality with lizards. Dispersed seeds under heterospecific shrubs showed higher seedling survival than those under conspecifics in all localities, especially the year with higher rainfall. Our findings support that the movement of seeds to nurse shrubs by lizards is the most important component of the seed dispersal process in the only remaining locality where both species coexist.

Keywords *Daphne rodriguezii* · Mutualism disruption · *Podarcis lilfordi* · Saurochory · Seed ingestion · Seed size · Seedling emergence and survival

Introduction

Despite long recognition that dispersal effectiveness of a given frugivore has two components (i.e. the quantitative, which tells us the number of seeds dispersed, and the qualitative, which inform us on the quality of the seeds dispersed and of the sites where seeds have been deposited), the qualitative component has received far less attention (Schupp 1993; Schupp and Fuentes 1995; Jordano 2000). This is probably because studying the quality of dispersal involves examining variables somewhat more difficult to measure such as: (1) effect of fruit selection, (2) influence of seed treatment in the disperser's guts on germination and (3) patterns of seed deposition in

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different microhabitats, and subsequent seedling recruitment and survival. In the case of mutualistic disruptions, the loss of seed dispersers directly influences the dispersal effectiveness and, consequently, the regeneration of those plant species that strongly depend upon them (Dinerstein and Wemmer 1988; Rodríguez-Cabal et al. 2007; Babweteera et al. 2007). Thus, to adequately assess how plant species and communities are affected by such disruptions, we need to know the importance of each one of those components affecting the dispersal process (Howe and Miriti 2004).

Frugivores may exert selection on different fruit traits, either morphological or physiological (e.g. size, colour, pulp–seed ratio, nutritional content). Within a species, the major determinants of fruit and/or seed-size selection depend on the capacity of handling and/or swallowing for each frugivore (Jordano 2000 and references therein). For instance, such selection has been reported in several studies, either for small (e.g. Jordano 1995; Alcántara et al. 1997; Riera et al. 2003) or large fruit and/or seeds (e.g. Courtney and Sallabanks 1992; Wheelwright 1993; Stanley et al. 2002). In fact, seed size determines the seed fate during subsequent post-dispersal stages (e.g. Alcántara and Rey 2003; Gómez 2004; Pizo et al. 2006; Martínez et al. 2007) and, thus, the selection for fruit and/or seed size can have important consequences for plant regeneration. Despite this, no studies have yet considered fruit selection as component of seed dispersal quality.

Moreover, the treatment that seeds experience within the disperser's digestive tract directly influences the capacity and speed of seed germination (Traveset et al. 2007 and references therein). Either pulp removal (i.e. de-inhibition effect) and/or the degree of chemical or mechanical coat scarification of ingested seeds are mechanisms underlying the germination of fleshy-fruited plants. In general, seed germination patterns depend on traits directly related to seed size, such as seed coat thickness and permeability and/or retention times inside digestive tract of dispersers (Traveset et al. 2007; Traveset et al. 2008). For endozoochorous plants, therefore, it is important to determine to what extent the process of seed ingestion affects the capacity and rate of germination of ingested seeds.

Finally, the patterns of seed deposition (which determine the seed germination patterns and,

ultimately, those of seedling recruitment) can be importantly influenced by abiotic factors, which actually have the potential to neutralize or enhance the previous disperser's effect on those variables (Schupp 1993; Schupp and Fuentes 1995; Schupp 2007). This is clear, for instance, in arid and semiarid environments, where the presence of nurse plants affects the spatial structure of plant populations; this is because endozoochorous species have higher probability of arrival there, and at the same time such microhabitats may constitute 'safe sites' for seedlings (Wenny 2001). Paraphrasing Howe and Miriti (2004), 'seed dispersal matters' when seedlings from dispersed seeds have a better fate than those remaining under the mother's canopy (e.g. Rey and Alcántara 2000, but see Lázaro et al. 2006; Montesinos et al. 2006). Nevertheless, sites are not intrinsically 'safe' or 'not safe' (sensu Schupp 1993), but depend on a broader spatio-temporal context (Schupp 2007).

In this study, we assess the importance of the dispersal phase, relative to early regeneration phases in *Daphne rodriguezii*, a threatened endemic shrub from Menorca Island (Balearic Islands, W. Mediterranean) and a small islet, Colom Islet, nearby. This species is a perfect model for this purpose because of its narrow interaction with an endemic lizard *Podarcis lilfordi*, its only seed disperser (Traveset and Riera 2005). In fact, strong dependences between species are particularly common in islands (Vitousek et al. 1995), leading to a greater vulnerability of these ecosystems to mutualistic disruptions caused by different types of disturbances (Traveset and Richardson 2006). In the present case, this frugivorous lizard became extinct from the two larger Balearic Islands, Mallorca and Menorca ca. 2000 years ago, presumably after the introduction of alien carnivores (Bover and Alcover 2008). Nowadays, the lizard only survives in surrounding islets of these two islands, and thus, the interaction between *D. rodriguezii* and *P. lilfordi* is only found in Colom Islet. In a previous study, Traveset and Riera (2005) attributed the differences in demographic structure and seedling distribution among localities to the presence or absence of lizards. Here, we disentangle which one of the components of the seed dispersal effectiveness is more important for the regeneration of *D. rodriguezii* and how they may affect plant regeneration after disperser loss. Specifically, our objectives were:

(1) to determine the probability of fruit removal and examine if this depends upon fruit/seed size, (2) to evaluate how seed passage through lizards' guts, seed size and the specific microhabitat where lizards deposit seeds, directly or indirectly influence seedling emergence and survival and (3) to know if seedling performance differs between the locality with lizards and those without them.

Materials and methods

Study species

Daphne rodriguezii Teixidor (Thymelaeaceae) is a small evergreen shrub, endemic to Menorca Island (Balearic Islands, W Mediterranean Sea). Its localities are scattered along the NE coastline, ranging in size from several dozens to <300 individuals. By far, the largest locality is found on Colom Islet (of ca. 60 ha. and located ca. 200 m east to Menorca), where nearly 20,000 individuals have been recorded and which shows the highest regeneration (Traveset and Riera 2005).

Daphne rodriguezii bears ripe fruits during May through June, being one of the few species with available fleshy-fruits at this time of year (pers. obs.). Fruits are orange–red drupes, the pulp of which bears high contents of phenolic compounds. Previous evidence strongly suggests that *P. lilfordi* Günter (Lacertidae) is its only seed disperser as (1) we have never observed either birds, human-introduced lizards or carnivores on the plants and/or consuming *D. rodriguezii* fruits, (2) we have only observed scats containing *D. rodriguezii* seeds in Colom Islet (Traveset and Riera 2005; pers. obs.) and (3) current and presumably 'relictual' distribution in Menorca island (i.e. located within 500 m of the coast, where lizards are usually more abundant) agrees with that of other lizard-dispersed plants of the Balearic Islands (Traveset 1999; Riera et al. 2003; pers. obs). Under controlled conditions, seeds from lizards' scats have a similar viability to those collected and depulped directly from plants (Traveset and Riera 2005). Seeds within the pulp do not emerge (at least during a period of 2 years; Santamaría et al. 2007), which further points out to the importance of lizards in the de-inhibition effect (Traveset et al. 2007). Post-dispersal seed predation (mostly by rodents) is consistently high across years and localities (Traveset and Riera 2005). Moreover, several ant species (e.g. *Messor structor*, *M. bouvieri* and *Crematogaster scutellaris*) have been observed removing seeds (Traveset and Riera 2005; pers. obs.), but we do not consider that they act as seed dispersers as (1) they are mainly granivorous and (2) we have never observed *D. rodriguezii* seedlings around occupied or abandoned ant nests. We have occasionally observed beetles (e.g. *Pimelia criba*) consuming the fruit pulp, leaving the seeds intact and at very short distances from the mother plant.

Study sites

We studied the main five localities of *D. rodriguezii*, four in Menorca Island (Porter, Mesquida, Favàritx and Pudent) and the one that in Colom Islet (see Traveset and Riera 2005). The habitat of *D. rodriguezii* is coastal shrubland, and the predominant vegetation of the community consists of *Phillyrea latifolia* subsp. *media*, *Pistacia lentiscus* and *Erica multiflora*. Differences among localities exist in vegetation composition and structure (Traveset and Riera 2005). While rainfall also varies across localities (range: 434–590 mm; CV: 20.0; data from the National Meteorological Institute), a higher variability exists among years (range: 373–684 mm; CV: 27.5). In terms of average rainfall over the last 20 years, the annual rainfall for the period 2001–2004 was considered to be 'normal' (ca. 615 mm, mean for the five localities) falling in the first quartile of that range; in contrast, 2000 and 2005 were dry years (ca. 405 mm, mean for the five localities). Most rain falls in autumn and winter (i.e. from September to February), as is typical in the Mediterranean climate.

Correlation between fruit and seed traits

During the springs of 2003–2004, we haphazardly collected ripe fruits from a minimum of 10 individuals from each of the five localities. All fruits were collected during one field survey in the mid-fruiting season (mid-June), when unripe fruits, ripe fruits and lizard-ingested seeds (only in Colom Islet) were available. These fruits were taken to the laboratory, where we measured fruit and seed diameters (to the nearest 0.01 mm) and weight (to the nearest 0.1 mg).

Data were also available for two more years, 2000–2001, from a previous study (Traveset and Riera 2005). Fruit and seed diameters (Pearson correlation: $r = 0.316$, $n = 981$, $P < 0.0001$) and fruit and seed mass ($r = 0.108$, $n = 981$, $P < 0.001$) were weakly correlated between them; pulp mass was strongly correlated with fruit mass ($r = 0.842$, $n = 981$, $P < 0.0001$), but not fruit mass with diameter ($r = 0.270$, $n = 981$, $P < 0.0001$) and mass of seeds ($r = 0.290$, $n = 981$, $P < 0.0001$). Despite neither diameter nor mass of seeds were good predictors of those traits which presumably select lizards (i.e. diameter and mass of fruits and pulp mass), we used seed diameter (rather than weight) as the potential variable subject to preference by dispersers, since that variable is that most likely constraining ingestion by frugivores (Herrera 2002). This variable was used to estimate the probability of seed removal relative to seed size (see "Data analysis" section).

Exclusion experiments

In order to asses the percentage of fruit removal by lizards (i.e. quantitative component of seed dispersal), we conducted a selective exclusion experiment on Colom Islet. We set up a total of 12 plots that included one or two D. rodriguezii fruiting adults and counted the total ripening fruits on them; we set the plots on 31 May to 2 June 2003, before fruit ripening, and left them throughout the entire dispersal period. Plots were divided into three groups which were assigned to different treatments: (a) predator-exclusion (we fenced an area of ca. 5 × 5 m by means of wire netting of 3 cm mesh size and 1 m tall) which allowed the passage of lizards but no rodents, (b) seed predator and lizard-exclusion (same as previous treatment but the fence consisted of a 1 m tall vinyl sheet) and (c) the control group; plots had the same area as the exclusion plots but were not fenced. In order to ensure effectiveness of (a) and (b) exclusions, we dug a 15-cm trench around the exclusion perimeter to bury the wire netting and vinyl sheet. Moreover, we pruned away those branches surrounding the exclusion perimeter (up to 15 cm) to avoid lizards and/or rodents climbing or jumping from adjacent shrubs. These exclusions were open from above, as we expected negligible seed removal by birds (Traveset and Riera 2005). We expected that fruits were rapidly consumed after ripening on Colom

Islet, so we revisited them on 11 June 2003 to record the number of fruits remaining on plants. On 26 May 2004, we recorded the number of new emerged seedlings in each plot. Since this species does not have a dormant seed bank (Traveset and Riera 2005), new emerged seedlings were from the previous year.

Fruit selection and ingestion effect on seedling emergence and survival

In order to determine whether there is any kind of seed-size selection by lizards and how seed treatment in their digestive tracts affects seedling emergence and/or survival, we collected lizard scats containing seeds of D. rodriguezii (sample sizes shown in Table 1) in Colom Islet. As lizard scats are more easily detectable on stone walls (Traveset and Riera 2005), we searched for them by running transects (a total of 100 m long) during one field survey along the stone walls found within the area. We did so during the mid-fruiting season of 2004, when enough fruits had already been consumed by lizards (they eat the fruits rapidly as these become ripe; Traveset and Riera 2005). On the same day and in the same area, we also gathered fruits (Table 1) directly from plants which we manually depulped to obtain seeds. Observations performed in the previous year indicated that rats (common in the islet) consumed seeds from lizard scats with a similar probability with which they consumed non-ingested (depulped) seeds (Generalized Linear Model: $\chi_1^2 = 0.03$, $P = 0.859$). The diameters of both lizard-ingested and non-ingested seeds were measured to the nearest 0.01 mm. These seeds were kept under the same conditions (in the dark and at room temperature) until sowing time (see below). The effect of seed size on the probability of seed removal was additionally evaluated from data gathered in 2000, in the study by Traveset and Riera (2005), although it was not analysed there to test such effect; data on seedling emergence and seedling survival from that experiment were not used here, as it was not performed in field conditions.

The effects of (1) seed ingestion by lizards, (2) microhabitat and (3) seed size and their interactions, on seedling emergence and survival were simultaneously evaluated by sowing the Colom Islet seeds under natural conditions. Since lizards can remain below shrubs for long periods (Santamaría et al. 2007) and most D. rodriguezii adults grow under

Table 1 Summary of the sample size (SS) to measure seed removal and seedling emergence and survival for each year, locality and microhabitat

Year	Locality	Microhabitat	SS seed removal	SS seedling emergence	SS seedling survival
2000	Colom (C)		30		
	Colom (I)		50		
2004	Colom (C)		300		
	Colom (I)		240		
2003	Porter	*Daphne*		70 (7)	18 (4)
		Phillyrea		70 (7)	14 (6)
		Pistacia		70 (7)	20 (3)
	Mesquida	*Daphne*		100 (10)	46 (8)
		Phillyrea		100 (10)	59 (10)
		Pistacia		100 (10)	56 (10)
	Colom (C)	*Daphne*		100 (10)	55 (10)
		Phillyrea		100 (10)	72 (10)
		Pistacia		100 (10)	60 (10)
2004	Porter	*Daphne*		36 (4)	3 (1)
	Mesquida	*Daphne*		79 (8)	28 (7)
	Pudent	*Daphne*		80 (8)	15 (7)
	Favàritx	*Daphne*		100 (10)	25 (8)
		Phillyrea		100 (10)	14 (6)
		Pistacia		100 (10)	14 (5)
	Colom (C)	*Daphne*		100 (10)	43 (9)
		Phillyrea		100 (10)	44 (10)
		Pistacia		100 (10)	45 (10)
	Colom (I)	*Daphne*		80 (80)	30 (8)
		Phillyrea		80 (80)	32 (8)
		Pistacia		80 (80)	38 (8)

Numbers *without parenthesis* were the number of seeds and seedlings, and *within parenthesis* were the number of sites (see "Materials and methods" section). A minimum of 10 fruits from each of 10 individuals were collected from each locality. For seedling emergence, SS was the total of sowed seeds and the sowed sites. For seedling survival, SS was the total of emerged seedlings and the sites of emerged seedlings. For Colom Islet, we distinguish between non-ingested (C) and lizard-ingested (I) seeds

shrubs, we wanted to test for differences in seedling emergence and survival among nurse shrubs, information not previously obtained by Traveset and Riera (2005). On 2 December 2004, we sowed both lizard-ingested and non-ingested seeds in Colom Islet (Table 1), each including the three most representative microhabitats (physiognomically distinguishable patches of vegetation) where *D. rodriguezii* grows: under (1) conspecifics (*Daphne* hereafter), (2) *P. latifolia* (*Phillyrea*) and (3) *P. lentiscus* (*Pistacia*). We did not include the 'open' microhabitat as (1) we do not observe new emerged seedlings in this microhabitat (i.e. lizards likely disperse few seeds there) in the undisrupted population (pers. obs.) and (2) seedling survival is consistently very low there

(Traveset and Riera 2005). In each site, we established two plots (separated by 10 cm) in which we sowed 10 lizard-ingested seeds and 10 non-ingested seeds, respectively; all seeds were placed at a depth of 1 cm below surface and were protected with a cage (30 × 20 × 2.5 cm) of wire mesh to prevent seed predation. We recorded seedling emergence on 15 June 2005 and survival one year later (11 June 2006).

Seedling emergence and survival among localities

The patterns of seedling emergence and survival were compared between Colom Islet and the Menorcan localities by means of sowing experiments carried out in 2003 and 2004 (Table 1). In each locality, we

collected and measured seeds and subsequently sowed them in each respective source locality. The rather low fruit crop usually produced by *D. rodriguezii* prevented control for plant source in this experimental design. In 2003, we haphazardly chose 4–10 (depending upon fruit availability) sowing sites bearing the three microhabitats mentioned above (i.e. under *Daphne*, *Phillyrea* or *Pistacia*) in two Menorcan localities and in Colom Islet (Table 1). In 2004, the low fruit crop in the Menorcan localities (except Favàritx) precluded a repeat experiment using the same three microhabitats and, instead, we sowed seeds under conspecifics in Porter, Mesquida and Pudent (Table 1), the microhabitat where most *Daphne* seedlings are found (Traveset and Riera 2005). In all localities, the chosen sowing sites were near those used in the previous year. A total of 10 depulped seeds were planted in each site both years. Seedling emergence was recorded in June of the first year after sowing, and seedling survival was recorded a year later.

Data analysis

All statistical analyses were performed using Generalized Linear Models (GLIMs) and likelihood ratio test III, with the GENMOD procedure of SAS 9.1 statistical package (SAS Institute 2000). Data on the probability of fruit removal, on seed selection, and on seedling emergence and survival were fitted to binomial distributions and logit link functions. Unless otherwise indicated, average values are reported as mean ± standard error (±1 SE) throughout the text.

Plots in the exclusion experiments were treated as random and subject factors, whereas treatment was a within-subject fixed factor. Seed selection models included year and treatment (i.e. lizard-ingested vs. non-ingested seeds) as fixed factors. Repeated measures' designs were used for seedling emergence and survival, including treatment, locality and year as within-subject fixed factors (depending on the model) and site as subject random factor. We treated year as a fixed factor, with years considered as proxies of 'dry' and 'normal' years (see "Study sites" section); year was also included in the model only in the case of Colom Islet, the locality for which we had data on seedling emergences and survival patterns for all three microhabitats and for the two consecutive fruiting seasons. Due to the unbalanced data design (Table 1), we carried out different analyses to test for the effect of the different independent variables on seedling fate: (a) effect of ingestion (lizard-ingested vs. non-ingested seeds in Colom Islet), (b) effect of locality (differences among localities were tested separately for each year) and (c) effect of year (tested only for data from Colom Islet). In all models, microhabitat was included as an independent variable and seed size as a continuous covariable.

Results

Fruit removal and seed-size selection

By 11 days after exclusion experiment set-up, no fruits remained on those plants to which lizards had access (either in the predator-exclusion treatment or in the control plots). In contrast, plants not accessible to lizards and rodents retained 100% of fruits either in or under the canopy of mother plants. Moreover, a higher seedling emergence was found in plots with lizards and rodents excluded ($36.9 \pm 20.1\%$; $\chi_2^2 = 12.35$, $P = 0.002$) than in the other two treatments ($7.9 \pm 1.8\%$, $P > 0.05$).

The probability of seed removal by lizards differed between years (Table 2a): large seeds were more likely to be removed than small seeds in 2000; in contrast, seed removal in 2004 was independent of size (Fig. 1).

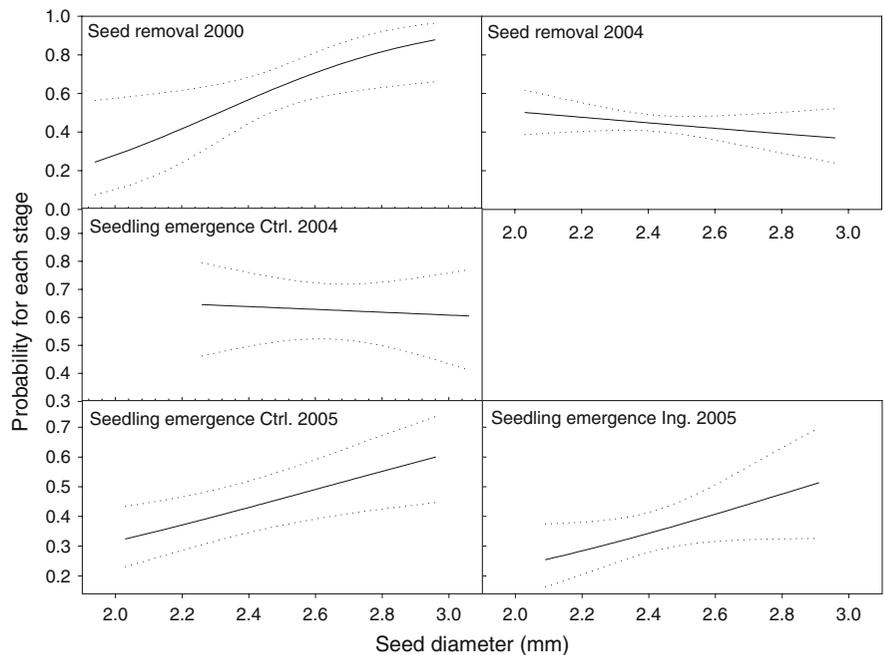Effect of seed ingestion on seedling emergence and survival

Seed passage through lizards' guts showed no significant effect on either seedling emergence or survival in Colom Islet, and this was consistent across microhabitats (Table 2b). Pooling ingested and non-ingested seeds, average germination was $41.1 \pm 3.3\%$ while average survival was $24.1 \pm 5.7\%$. However, the effect of seed size on seedling emergence seemed to vary depending on whether seeds had been ingested or not (Table 2b): large seeds had higher chances of seedling emergence than small seeds, but the effect was greater for non-ingested than for ingested seeds (Fig. 1). In contrast, seed size was not found to affect seedling survival (Table 2c).

Table 2 Results of the Generalized Linear Model analysis on (a) seed-size selection (during the fruit removal phase), (b, d) seedling emergence, (c) survival between treatments and (e) survival between years

Variable	d.f.	χ^2
(a) Seed-size selection—difference between years		
Year	1	9.57**
Seed diameter × year	2	7.60*
(b) Seedlings emergence—difference between treatments in 2005		
Seed diameter × treatment	2	7.10*
(c) Seedlings survival—difference between treatments in 2005		
Seed diameter	1	0.01[n.s.]
(d) Seedlings emergence—difference between years		
Seed diameter × year	2	5.80*
(e) Seedling survival—difference between years		
Microhabitat	1	3.21[n.s.]
Microhabitat × seed diameter	1	3.32[n.s.]

Data from the previous analyses came exclusively from Colom Islet. Independent variables were seed removal (a), year (a, d, e), treatment (b, c), microhabitat (b–e), seed diameter (a–e), and their two-way interactions among them. For each analysis, we show those independent variables included in the model with the lowest AIC score. Independent variables not shown in tables were not selected by the best model and, thus, they were non-significant ($P \gg 0.05$). Significance: *** $P < 0.001$, ** $P < 0.005$, * $P < 0.05$, [†] $P < 0.1$, [n.s.] $P > 0.1$

Fig. 1 Estimates of the predicted probability of seed removal by lizards and the seedling emergence, dependent upon seed diameter in Colom Islet. For seedling emergence in 2005, lizard-ingested (Ing.) and non-ingested seeds (Ctrl.) were plotted separately due to the different effect of seed size on seedling emergence between treatments (Table 2b). Mean is presented as *continuous lines* and errors (±1 CI) as *dashed lines*

The effect of seed size on seedling emergence (only with data from non-ingested seeds) varied slightly between the 2 years (Table 2d): it was not significant for seeds emerged in 2004, whereas it was positive for those emerged in 2005 (Fig. 1). These inconsistent results may be attributed to differences in seed diameter between years, seeds being somewhat larger in 2004 than in 2005 (2.70 ± 0.09 mm vs. 2.43 ± 0.09 mm, respectively: t test: $t = -20.7$, d.f. $= 600$, $P < 0.001$).

Differences among microhabitat and localities

Seedling emergence in 2004 was independent of the nurse species under which seeds were planted, and this effect was consistent across localities (Table 3a; $P \gg 0.05$ for 'microhabitat' and 'microhabitat × locality' effects). In contrast, seedling survival was higher under heterospecific nurse shrubs ($48.6 \pm 4.8\%$) than under *Daphne* ($33.2 \pm 7.1\%$; $\chi^2_2 = 6.76$, $P = 0.034$; Table 3b). The following year, data from Colom Islet and Favàritx consistently showed no effect of microhabitat on seedling emergence, and this time also a non-significant effect on seedling survival (Table 4a, b).

Regarding differences across localities in 2004, we found seedling emergence highest in Colom Islet and lowest in Porter (Table 3a; Fig. 2). Likewise, seedling survival was higher in Colom Islet (but also in Mesquida) than in Porter (Table 3b; Fig. 2). Overall, seed size showed no significant effect on either seedling emergence or survival ($P \gg 0.05$, for both variables). The only exception was found in Mesquida, where seedlings coming from small seeds had a marginally lower survival probability than large seeds $\log(P/1 - P) = -6.95x + 7.85$, $z = 14.06$, $P = 0.05$) compared to other localities (which did not differ; $P \gg 0.05$).

In 2005, Colom Islet showed higher seedling emergence but lower seedling survival than Favàritx (Table 4a, b; Fig. 2). Large seeds tended to germinate more than small seeds (only non-ingested seeds from Colom considered for this comparison; Table 4a, b).

Table 3 Results of the Generalized Linear Model analysis on (a) seedling emergence and (b) survival in 2004

Variable	d.f.	χ^2
(a) Seedling emergence in three microhabitats		
Locality	2	7.26*
(b) Seedling survival in three microhabitats		
Microhabitat	2	6.76*
Locality	2	11.21**
Seed diameter	1	0.00[n.s.]
Seed diameter × locality	2	10.59**

Independent variables were location, microhabitat, seed size and their two-way interactions among them. For each analysis, we show those independent variables included in the model with the lowest AIC score. Significance and symbols as in Table 2

Table 4 Results of the Generalized Linear Model analysis on (a) seedling emergence and (b) survival among three microhabitats, (c) seedling emergence and (d) survival under *Daphne* in 2005

Variable	d.f.	χ^2
(a) Seedling emergence in three microhabitats—two localities		
Locality	1	10.80*
Seed diameter	1	6.37*
(b) Seedling survival in three microhabitats—two localities		
Locality	1	3.92*
(c) Seedling emergence under *daphne*—five localities		
Locality	4	9.75*
Seed diameter	1	3.91*

Independent variables were location (a–e), microhabitat (a, b), seed diameter (a–d) and their two-way interactions among them. The locality of Porter was excluded from the analysis of seedling survival as all emerged seedlings died during that summer. For each analysis, we show those independent variables included in the model with the lowest AIC score. Significance and symbols as in Table 2
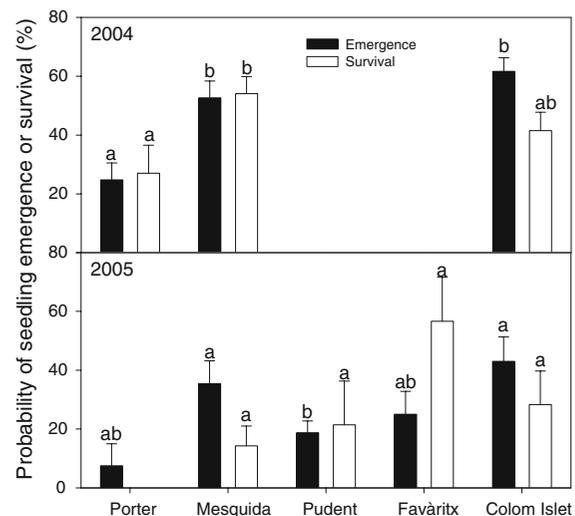

Fig. 2 Mean (±1 SE) percentage of seedling emergence and survival in each locality and year. For 2005, seedling emergence (and the differences among localities) was plotted only for the 'under *Daphne*' microhabitat. For a given year and variable (seedling emergence or survival), bars with *different letters* above differed significantly ($P < 0.05$) among localities. Multiple pair-wise contrasts were corrected by sequential Bonferroni (Rice 1989). For sample sizes per each locality and year, see Table 1

Survival was not affected by seed size. Finally, when comparing results from all localities considering only the 'under *Daphne*' microhabitat, we found that

seedling emergence was slightly higher in Colom Islet and Mesquida than in the other localities (Fig. 2; Table 4c); in contrast, differences in seedling survival did not differ across localities (Fig. 2; Table 4d).

Discussion

Effect of seed-size selection by lizards

Although small fruits may generally have greater chances of being successfully handled and swallowed by frugivores (Jordano 2000 and references therein), there are studies reporting preference for either large (e.g. Courtney and Sallabanks 1992; Wheelwright 1993; Stanley et al. 2002; Martínez et al. 2007) or small fruits and/or seeds (e.g. Jordano 1995; Alcántara et al. 1997; Riera et al. 2003). In *D. rodriguezii*, results on seed selection were inconsistent, as a higher probability of removal for larger seeds was only found for one of the two studied years. Seed size in *D. rodriguezii* is, on average, 2.47–2.50 mm (± 95% CI), a range that falls well within that of *P. lilfordi*'s gape size (7–11 mm, unpubl. data). Depending upon other food availability, lizards might show a stronger preference for large seeds during some years. Nevertheless, we expect seed-size selection to be low since (1) seed diameter was not a good predictor of either fruit or pulp mass (i.e. those traits presumably selected by lizards), (2) exclusion experiments showed that lizards removed most or all fruits from shrubs and (3) we cannot discard the possibility that seed selection by rats modified the observed patterns of seed selection exerted by lizards. Fruits in Colom Islet are usually consumed soon after ripening (pers. obs.). Data from more years would certainly be needed to know how consistent that selection is and under what circumstances it is strong or weak. To our knowledge, most studies on seed-size selection by frugivores in field conditions are performed during one or only a few reproductive seasons (but see Sallabanks 1992); therefore, it is still too soon to make generalizations about what the patterns are in other plant–frugivore systems and especially in plant–lizard seed disperser systems.

Even though differential preference for specific fruit and/or seed traits has been extensively evaluated, both among (Herrera 1984; Izhaki 2002) and within species (e.g. Jordano 1995, Alcántara et al. 1997), there is little information on how these traits can influence subsequent regeneration stages (i.e. seed-size conflicts; but see Alcántara and Rey 2003; Martínez et al. 2007). We found that, at least in 1 year, large seeds tended to have higher chances of emergence (although similar survival) than small seeds, especially the non-ingested seeds. We attribute the temporal inconsistency to differences in seed diameter between years; in other words, the advantages (better germination) of a greater seed size might appear after reaching a given threshold size. A lower probability of seedling emergence and survival for small seeds compared to large ones has also been reported for other Mediterranean species such as *Olea europaea* var. *sylvestris* (Alcántara and Rey 2003) and *Quercus ilex* (Gómez 2004). In general, these studies have been performed in only one locality and 1 year, which imposes an important limitation in terms of making any generalization. Clearly, spatio-temporal variation (a factor not usually included in the experimental designs of studies on seed size preference by frugivores) needs to be taken into account in future research if we want to assess whether the preference of seed dispersers on particular fruit traits have ecological and/or evolutionary implications for plant regeneration.

Seed ingestion by lizards versus seed deposition effects

Some studies of seed dispersal performed by only one species suggest that the success of this stage on plant regeneration is related to the establishment of new individuals in areas favourable for persistence in the long run (Dinerstein and Wemmer 1988; Rodríguez-Cabal et al. 2007; Babweteera et al. 2007). Besides being the only seed dispersal vectors of *D. rodriguezii*, lizards are important for depositing seeds intact and ready to germinate. Overall, the passage of seeds through lizards' guts was found neither to enhance nor inhibit its germination, which agrees with the general pattern found in a meta-analysis for reptiles (Traveset and Verdú 2002; but see Rodríguez-Pérez et al. 2005). Seed ingestion by lizards, however, appeared to diminish the effect of seed size on seedling emergence. A recent study has shown that both seed coat thickness and permeability are dependent on seed size, that they are modified when

passing through a frugivore's digestive tract, and that this modification may alter germination patterns (Traveset et al. 2008). In the case of *D. rodriguezii*, we hypothesize that ingestion by lizards reduces the coat thickness and/or increases the permeability proportionally more in small than in large seeds. In general, small seeds have usually longer retention times inside the digestive tract of dispersers than large seeds, which favours seed coat scarification (Traveset et al. 2007 and references therein). In the present system, however, this does not seem to be the case, as retention time is independent of seed size (Santamaría et al. 2007). In this latter work, the authors also found that larger seeds had higher chances of germination, but the effect of a larger size was reduced as retention time increased. In agreement with such results, we found that larger lizard-ingested seeds had proportionally lower chances of germination than non-ingested ones. As mentioned above, however, seed size may vary from year to year (depending on a variety of environmental factors), and this, in turn, may determine the effect it will have on seedling emergence and/or survival. Furthermore, the undisrupted locality showed proportionally higher rates of seedling emergence (regardless of whether seeds were ingested or not) which may result from the effect of factors unrelated to the presence of the seed disperser; for instance, (a) better climate, edaphology and/or vegetation structure and/or (b) higher plant fitness due to the larger population size in this locality, with presumably lower levels of inbreeding (Dudash and Fenster 2000). Certainly, more information is needed to support which one of the last hypotheses is responsible for the higher seedling emergence of the undisrupted population. Furthermore, lizards also remove the pulp around the seed (which is needed for germination to occur; Santamaría et al. 2007) but most non-dispersed seeds are depulped by either ants or beetles (Traveset and Riera 2005; pers. obs.). Thus, lizards do not seem as important in the de-inhibition effect as in other qualitative components of the seed dispersal process.

In cases of disruption of plant–animal interactions, dispersal is a key stage when seeds or seedlings have a lower performance under the canopy of conspecifics than in other microhabitats (Howe and Miriti 2004 and references therein). The seeds of *D. rodriguezii* deposited under mother plants showed lower seedling survival compared to those under heterospecifics, as

has also been reported in other short-term studies in the Mediterranean region (e.g. Rey and Alcántara 2000, but see Lázaro et al. 2006; Montesinos et al. 2006). However, a lower performance under conspecifics may not be consistent across years, as we found in this study and may depend upon specific environmental conditions. For example, plant–plant interactions can switch from being facilitative to competitive along a gradient of increasing rainfall (Brooker et al. 2008). Moreover, the competitive and facilitative response and/or tolerance to stress may be also dependent on each species-specific interaction. In the case of *D. rodriguezii*, although we had data for only 2 years, the temporal differences found in seedling survival might be attributed to rainfall. The first experimental emergences (i.e. in 2004) showed differences among microhabitats, which coincided with a year with average rainfall. In contrast, rainfall was much lower the following year, when all microhabitats were similarly poor sites for seedling survival. These findings are in accordance with the concept of 'safe site', by which the suitability of sites for dispersed seeds is context dependent. In other words, sites are not intrinsically 'safe' or 'unsafe' (binary concept; sensu Schupp 1993) but vary continuously—in space and time—in the likely fate of recruits (Schupp 2007).

In short, of the components of seed dispersal effectiveness studied in *D. rodriguezii*, the movement of seeds that lizards perform to nurse shrubs appears to be the most important for plant regeneration. Although seed size was selected by lizards in some years, this may not translate into subsequent regeneration stages, since seedling survival of ingested seeds was independent of seed size. Likewise, passing through the digestive tract of lizards was not critical for seed germination, although it tended to reduce the effect of seed size: larger non-ingested seeds tended to germinate more than large ingested seeds. The pattern of seed deposition seems to affect seedling survival in the recipient nurse shrubs. Nevertheless, this probably depends on climatic conditions, such as the amount of rainfall in each year: in a favourable year (with average rainfall), seedlings located under conspecifics showed lower survival than under heterospecifics, whereas in a dry (stressful) year seedling survival was independent of nurse shrub. Our findings support previous predictions by Traveset and Riera (2005) that if *P. lilfordi*

disappeared from the unique locality (Colom Islet) where this lizard coexists with *D. rodriguezii*, this would promote a decline in the capacity of seeds to reach the best sites for seedling establishment.

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