

Secondary seed dispersal systems, frugivorous lizards and predatory birds in insular volcanic badlands

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Summary

1. Secondary seed dispersal occurs whenever a seed is dispersed in two or more different dispersal events, so that different dispersal agents (e.g. animal frugivores or invertebrates) contribute to different events. Three secondary seed dispersal systems, in which lizards and predatory birds participate, are studied in Lanzarote (Canary Islands).

2. Seeds from all three plant species studied (*Lycium intricatum*, *Rubia fruticosa* and *Asparagus nesiotus*) were found with the remains of lizards (*Gallotia atlantica*) when they appeared inside shrike (*Lanius meridionalis*) and kestrel (*Falco tinnunculus*) pellets. This suggests that these two avian predators might secondarily disperse these plants by incidentally ingesting seeds when they prey upon the frugivorous lizards.

3. *Lycium* and *Rubia* seeds dispersed by *Falco* had significantly thinner seed coats compared to the other treatments (*Gallotia* and *Lanius*) and to uningested seeds. Correlated with this result, seed hardness increased from *Lycium* (the softest coat), to *Rubia* (intermediate values), to *Asparagus*.

4. Seed viability was high in all three species, although both viability and germination were significantly reduced in seeds of *Lycium* and *Rubia* dispersed by *Falco*.

5. The seed distribution was markedly different, *Gallotia* being an important disperser in the open ground microhabitats, *Lanius* in hillocks and *Falco* in hills. Because all three plant species were present in all these microhabitats, our observations suggest that the dispersal of these plants might be associated with the differential use of the habitat by the different dispersers.

6. Synthesis: Contrary to some long-distance seed dispersal (LDD) paradigms, the complex seed dispersal systems we describe are common and affect an important number of seeds that are moved each year to particular microhabitats. Finally, the simultaneous ecological factors analysed in this study support the hypotheses that diplochory by double endozoochory could have played a more important role in LDD events than is currently recognized, both in recent volcanic areas (lowlands) and also probably in the colonization of other subtropical islands.

Key-words: Badlands, Canary Islands, diplochory, endozoochory, frugivorous lizards, island plant colonization, long-distance seed dispersal, seed coat and hardness, seed distribution, seed viability and germination.

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Introduction

The intervention of two or more different agents in the dispersal of a seed is defined as diplochory (see review of Vander Wall & Longland 2004) and this multi-step ecological process is considered to be important in the

reproductive cycle of some plants (Chambers & MacMahon 1994). Such a process functions because the combination of two or more dispersers can disproportionately increase the benefits of seed dispersal, for instance by reducing seed mortality and by providing long-distance seed delivery.

Secondary seed dispersal systems are widely variable because the potential combinations of dispersal agents (both abiotic and biotic) are large. Thus, secondary seed dispersal sometimes involves abiotic factors (wind, water or ballistic systems) as primary dispersal agents, and scatter-hoarding vertebrates (Vander Wall 2002) or invertebrates (Espadaler & Gómez 1996; Pizo *et al.* 2005) as secondary dispersers. In other cases, primary seed dispersal includes a first process of endozoochory, by mammals and birds, and sometimes other vertebrates (Forget & Milleron 1991; Wenny 1999), followed by secondary dispersal by invertebrates such as ants (Kauffmann *et al.* 1991; Levey & Byrne 1993) or beetles (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Andresen 2001).

Most of the seed dispersal systems in which a vertebrate frugivore participates include only one seed digestion event (Ridley 1930). However, secondary seed dispersal can be more complex, especially if a second seed digestion process is mediated through frugivore–predator interaction. This occurs whenever predatory vertebrates prey upon frugivores that have already consumed fruits, thus ingesting the entire seed load contained within the digestive tract of the prey. Although this phenomenon has rarely been described (Damstra 1986; Hall 1987; Dean & Milton 1988; Nogales *et al.* 1996), only a few studies carried out in insular environments have partially attempted to interpret it in an ecological context (Nogales *et al.* 1998, 2002). These studies, in which an endemic lizard (*Gallotia atlantica* Peters & Doria) performed the first dispersal event, focused only on a single plant species (*Lycium intricatum* Boiss., Solanaceae) on a small island (Alegranza, Canaries), and showed that successful seed survival can be affected by the secondary dispersal by avian predators that prey upon the frugivorous lizards (shrike: *Lanius meridionalis* Temminck, or kestrel: *Falco tinnunculus* Linnaeus). To date, only some factors such as viability and germination before and after gut treatment by the different dispersers were studied. However, in this complex ecological process, where seeds undergo a double gut-processing, other important factors, such as coat thickness, seed hardness and seed rain in different microhabitats, have not been evaluated. The study of these factors within such a diverse assemblage of interactions will permit a more complete interpretation of the ecological role of this secondary seed dispersal system. An additional element for evaluating the importance of these secondary seed disperser events is whether other fleshy-fruited plant species present in more complex insular habitats are also involved.

The scarification of seeds by the long residence times in guts of more than one vertebrate disperser can affect

seed viability, and thus, the probability of long-distance dispersal (hereafter LDD, *sensu* Nathan 2006). In this respect, seed-coat thicknesses can sometimes account for the diverse effects of different frugivores on seed germination (Gardener *et al.* 1993; Traveset *et al.* 2001; Nogales *et al.* 2005). Seed germination may also be influenced by other factors, such as seed hardness or robustness, which might determine to some extent the strength needed for the seedling to break the seed coat. However, the demographic effects of these traits are still poorly understood.

Traditional studies on island biogeography have mentioned the importance of LDD events in insular colonization processes (e.g. Ridley 1930; Carlquist 1967). The current LDD paradigm suggests that these kinds of ecological processes are very difficult to predict and to document in time and space (Greene & Johnson 1995; Higgins & Richardson 1999; Clark *et al.* 2001). However, some studies of LDD using mechanistic models (see Nathan *et al.* 2002; Nathan & Katul 2005 and references therein) or DNA-based genotyping (Jordano *et al.* 2007) have successfully documented both frequency and distance of these events.

The present work deals with LDD systems in oceanic island environments in which two dispersers participate (double endozoochory). Furthermore, it is an environment where volcanic eruptions take place with relative frequency, on a geological time scale, and the colonization of a new lava field by organisms such as fruiting plant species is clearly a very important, albeit poorly known process. Older volcanic zones function as genetic sources, where biological material may spread and colonize the new lava fields when they cross the older zones. However, knowledge of the functioning of the mechanisms that make these colonization events possible is still scant.

This paper attempts to evaluate the effectiveness (*sensu* Schupp 1993) of secondary seed dispersal by two predatory birds (the shrike and the kestrel) in three shrubs (*Lycium intricatum*, Solanaceae; *Rubia fruticosa* Aiton, Rubiaceae; and *Asparagus nesiotus* Svent., Convallariaceae) by examining several factors simultaneously. The specific aims of this research are: (i) to document secondary dispersal events by predatory birds by examining the correlation of prey carcasses with fruiting plant seeds; (ii) to evaluate the relative importance of primary dispersers (lizards) and secondary seed dispersers (shrikes and kestrels) in the dispersal events of the three fruiting plant species; (iii) to examine changes in seed coat thickness and hardness after two vertebrate gut-passage events; (iv) to study the gut effect of reptiles and birds on seed viability and germination, (v) to assess the differential seed distribution caused by the different seed dispersers and evaluate the presence of these fleshy-fruited plant species according to their respective habitat use; and (vi) to quantify the movement distances of the seed dispersers in order to better understand the potential magnitude of these LDD processes, and their potential incidence in the

colonization of fleshy-fruited plant species in recent lava flows (badlands) of volcanic islands.

Methods

STUDY AREA

The Canary Archipelago is made up of seven main volcanic islands located between 27 to 29° N and 13 to 18° W, and less than 100 km off the north-western coast of Africa. This study has been carried out in a xeric badland (50–100 m a.s.l.) in the north of Lanzarote, one of the driest easterly islands of the Canaries. This area is known as Malpaís de La Corona and it is one of the most extensive badland areas (c. 18 km²) in the entire archipelago. The age of this island has been estimated as 15.5–5.0 million years, while a relatively recent volcanic episode originated on this badland (about 21 000 years ago; Carracedo *et al.* 2003). The maximum elevation of Lanzarote is 671 m a.s.l. and its climate is semi-arid, being strongly influenced by the proximity of the African continent. Mean rainfall in this badland is 157.8 ± 80.6 mm per year ($n = 19$ years; between 1973 and 2002) and the annual mean temperature is 21 ± 0.60 °C ($n = 9$ years; period: 1989–97). Vegetation consists of sparse xerophytic shrub composed mainly of species such as *Euphorbia regis-jubae* Webb & Berthel., *E. balsamifera* Aiton, *Launaea arborescens* (Batt.) Murb., *Kleinia neriifolia* Haw., *Lycium intricatum*, *Rubia fruticosa*, *Asparagus nesioties* and scarcer and locally distributed *A. arborescens* Willd. This badland was chosen because most of the native fleshy-fruited plant species of the island are present in the area. Therefore, this area provides an opportunity to understand how this complex multi-dispersal process could function. These ecological events could have been decisive in the colonization of such areas by fleshy-fruited plant species in the geological past. During these colonization episodes, although the participation of short-distance dispersers (generally lizards and passerines in oceanic islands – Valido & Nogales 1994; Olesen & Valido 2003; Nogales *et al.* 2005) is frequent, LDD events of seeds are also presumably important (Moore 1999; Nogales *et al.* 2001).

EXPERIMENTAL PROCEDURES

Fieldwork was conducted over four years (springs of 2002–05) in two sites (1.6 km apart) in Malpaís de La Corona: ‘Las Tabaibitas’ (100 m a.s.l.) and ‘Peñas de Tao’ (140 m a.s.l.). A total of 200 fruits (< 10 per plant) were directly collected from each species of plant (controls); a total of 566 lizard droppings and 713 pellets (342 from shrikes and 371 from kestrels) were also obtained during the 4-year study. These samples were collected in both study sites, over an area of 4000 m², to minimize the mother plant effect. They were used to evaluate the effect of gut passage by the different disperser agents on seed viability and germination.

Each dropping and pellet was stored independently, and seeds were manually extracted and counted. Damaged and undamaged seeds were classified using a stereomicroscope (10× magnification) according to the visual state of seeds. Seeds were assigned to different treatments on the basis of their origin: (i) control seeds, (ii) from lizard droppings, (iii) from shrike pellets, and (iv) from kestrel pellets. Interaction effects between the apparitions of seeds linked to the presence of lizards in the pellets from each predatory bird were studied in the case of the three plants studied.

Viability was assessed for 50 seeds from each treatment, except for the *Asparagus–Gallotia–Falco* interaction due to infrequent occurrence ($n = 2$ pellets). The bioindicator 2,3,5 triphenyl-tetrazolium chloride (TTC) was used to determine viability. In addition, a germination experiment for each treatment was performed in a greenhouse over a period of 6 months (1 October 2003 to 31 March 2004). A total of 200 seeds were planted for most treatments; each seed was sown 5 mm deep independently in a 4-cm² pot ($n = 230$ pots per tray) containing a standard substrate (50% turf and 50% culture soil). The experiment was carried out at Tagoro (Tenerife Island; 300 m a.s.l.), with a night/day cycle similar to that found in the study area. Pots were watered every 2 days and germination date was noted when any seedling part emerged above the soil surface.

Seed coat thickness measurements were gathered from approximately 15 seeds ($n = 10$ repeated measurements per seed) from each treatment, by means of a dissecting microscope connected to a computer using Image Pro-Plus vs. 4.5.1.2.2 software (Media Cybernetics, Inc., USA). Seed hardness was measured by using an electronic press (Zwick/Roell Z100; Ulm, Germany), with the help of the ‘testxpert Machine’ program. Unfortunately, this machine could only be used in the case of *Asparagus* seeds, as *Lycium* and *Rubia* seeds were too flexible to obtain the breaking point. Nonetheless, from the point of view of seed consistency, we can appreciate a clear cline of decreasing hardness: *Asparagus* > *Rubia* > *Lycium*.

Seed distribution was studied by counting dispersed seeds in the three microhabitats previously classified: (i) open ground (flat rocky places with shrubby vegetation), (ii) flat-topped volcanic hillocks with shrubby vegetation (small promontories; mean size: 31.3 ± 34.6 m², height: 2.67 ± 1.69 m, $n = 29$), and (iii) small volcanic hills (promontories higher and larger in size than the flat-topped volcanic hillocks; mean size: 401.2 ± 383.2 m², height: 11.7 ± 2.75 m, $n = 10$). For this sampling, randomly placed (0.5 × 0.5 m²) plots were used, analysing all droppings and pellets found (400 plots in the open ground, 151 in the hillocks and 37 in the hills). Furthermore, in the same plots, we counted the number of the three fleshy-fruited plant species studied in this work. Sampling of droppings, pellets and fleshy-fruited plants were carried out during two springs (2004 and 2005).

Lastly, to evaluate potential long-distance seed dispersal carried out by the two secondary dispersers, direct observations on flights (estimation bias: ± 25 m) in the badland were made during spring 2005, using a detailed GPS-supported map of the study area in order to reduce bias in the data. Only those flights in which the departure site and the landing site could be observed were considered. According to this, the duration of most flights was short, ranging approximately from a few seconds to a few minutes. The sampling location was often changed in order to perform not more than three observations per individual. Furthermore, these data were supported by studying movements of seeds carried by these predators in a border zone of Malpaís de la Corona (Llanos de Órzola, easternmost edge of the badland) where land has been extensively modified by man through cultivation. In this area, old volcanic formations (hillocks and small hills) are also present, and they are actively used by the two predatory birds which frequently fly between the badland area and formations in this modified landscape. In this area, one of the three studied plants is especially scarce with respect to their abundance in the badland area (*Asparagus*: 0.6 ± 1.6 vs. 27.7 ± 28.3 plants per ha, respectively) and we did not record any fruiting throughout the last 6 years in this area. Therefore, this framework is ideal to evaluate minimum long-distance seed dispersal movements from the nearest population of *Asparagus* located in the badland border.

STATISTICAL ANALYSIS

We tested the association between the presence of *Lycium*, *Rubia* and *Asparagus* seeds and lizard remains in shrike and kestrel pellets, by Likelihood ratio tests (*G*-tests). The number of seeds found in droppings and pellets of the dispersers was analysed by a Kruskal–Wallis test. Differences in seed coat thickness among the different treatments were analysed by ANOVA. The average of the 10 measurements per seed was used in the analysis. Seed viability and germination of uningested and ingested seeds were evaluated by Likelihood ratio tests. As we performed multiple independent significance tests, we used the Bonferroni correction ($0.05/k$) to avoid inflated Type I error rates. The speed at which seeds germinated was evaluated by applying Kolmogorov–Smirnov tests. Residuals of a contingency table analysis (disperser \times microhabitat) were evaluated in order to assess if each disperser favoured the arrival of seeds to particular microhabitats. Seed movement by both predatory birds was calculated by a Mann–Whitney test. However, in order to avoid potentially misleading averages, a ‘dispersal kernel’ analysis was performed, ranking the flight distances carried out by both predatory birds (10–100 m, 101–200, 201–300, etc.) and assigning their respective frequency of displacement; for this statistical analysis, a likelihood ratio test was performed. Statistical analyses were performed using the SPSS statistical package (version 14.0) (SPSS 2005).

Table 1. Number of pellets for both predatory birds. Results of association analysis (likelihood ratio tests) of the plant species (*Lycium*, *Rubia* and *Asparagus*) seeds and *Gallotia atlantica* remains in regurgitated pellets from *Lanius meridionalis* ($n = 342$ pellets) and *Falco tinnunculus* ($n = 371$ pellets) on Malpaís de La Corona (Lanzarote, Canary Islands)

	<i>Lycium</i>		<i>Rubia</i>		<i>Asparagus</i>
	<i>Lanius</i>	<i>Falco</i>	<i>Lanius</i>	<i>Falco</i>	<i>Lanius</i>
Seeds + lizards	161	39	57	27	34
Seeds alone	14	3	5	0	1
Lizards alone	126	229	225	241	253
None	41	100	55	103	54
G_1	17.93	12.55	5.46	18.36	7.0
P	< 0.001	< 0.001	0.019	< 0.001	0.008

Results

SECONDARY SEED DISPERSAL

We found a statistically significant association between the presence of seeds and lizard prey remains in the pellets of both *Lanius* and *Falco* (Table 1). This suggests that the two predatory bird species only dispersed seeds after consuming lizards that had eaten fruits for the three plants studied. We never recorded either *Lanius* or *Falco* feeding directly upon fruits. Furthermore, in all *Falco* pellets where *Asparagus* seeds were present ($n = 2$), lizard remains were also detected.

SEED MOVEMENTS, VERTEBRATES AND PHENOLOGY

From the total number of seeds found in the *Gallotia* droppings and in the *Lanius* and *Falco* pellets, 8752 (74.7%) were *Lycium*, 2641 (22.5%) were *Rubia* and 321 (2.8%) were *Asparagus* seeds. Regarding the vertebrates, *Lanius* carried 8019 (68.5%) of the total seeds, *Gallotia* 3351 (28.6%) and *Falco* 344 (2.9%). *Lycium* and *Rubia* seeds were generally found at a higher frequency and mean number per sample than were those of *Asparagus* in both *Gallotia* droppings and in *Lanius* and *Falco* pellets (Table 2). Shrikes clearly carried the greatest number of *Lycium* seeds compared to the other two dispersers (Kruskal–Wallis test, $\chi^2 = 117.37$, d.f. = 1, $P < 0.001$). However, a similar mean number of *Rubia* seeds were counted in lizard droppings and shrike pellets.

Lycium seeds were basically dispersed at the beginning of spring (March), gradually decreasing in number in the following months. Seeds from *Rubia* were mainly dispersed in April and May. *Asparagus* seeds reached maximum dispersal in May.

Table 2. Seeds from the different plant species (*Lycium*, *Rubia* and *Asparagus*) found in droppings and pellets from lizards, shrikes and kestrels in Malpais de La Corona (Lanzarote, Canary Islands)

	Total seeds found			Seed number per dropping or pellet (mean ± SD)			Percentage of droppings or pellets where at least one seed was recorded			Percentage of undamaged seeds			Number of droppings or pellets analysed
	<i>Lycium</i>	<i>Rubia</i>	<i>Asparagus</i>	<i>Lycium</i>	<i>Rubia</i>	<i>Asparagus</i>	<i>Lycium</i>	<i>Rubia</i>	<i>Asparagus</i>	<i>Lycium</i>	<i>Rubia</i>	<i>Asparagus</i>	
<i>Gallotia</i>	1392	1717	242	2.46 ± 5.89	3.03 ± 4.37	0.43 ± 0.64	27.7	49.1	35.9	100	99.3	99.2	566
<i>Lanius</i>	7147	795	77	20.90 ± 38.40	2.32 ± 7.19	0.23 ± 0.82	51.2	18.1	10.2	99.7	100	100	342
<i>Falco</i>	213	129	2	0.57 ± 2.77	0.35 ± 1.69	0.0053 ± 0.073	11.3	7.3	0.5	99.5	99.2	100	371

Table 3. Seed coat thickness of *Lycium*, *Rubia* and *Asparagus* recorded in the different treatments (lizards, shrikes and kestrels) on Malpais de La Corona (Lanzarote, Canary Islands); *n*: number of seeds

	Seed coat in µ (mean ± SD)						Percentage reduction (cf. control)		
	<i>Lycium</i>	<i>n</i>	<i>Rubia</i>	<i>n</i>	<i>Asparagus</i>	<i>n</i>	<i>Lycium</i>	<i>Rubia</i>	<i>Asparagus</i>
Control	149.03 ± 15.39	14	80.99 ± 6.25	15	56.03 ± 4.21	14	–	–	–
<i>Gallotia</i>	136.17 ± 16.66	14	53.09 ± 4.19	15	52.26 ± 5.75	11	8.7	34.5	6.7
<i>Lanius</i>	134.77 ± 14.23	14	52.63 ± 5.58	15	53.31 ± 5.32	10	9.6	34.2	4.9
<i>Falco</i>	125.13 ± 11.07	15	46.33 ± 4.79	15	48.37	1	16.1	57.2	13.7

SEED GUT TREATMENTS

External visual damage of seeds from the three different plant species was low; the percentage of ingested seeds that were undamaged was over 99% (Table 2). In general, decreasing differences were observed in seed coat thickness after the seeds were consumed by the different dispersers compared to control seeds (Table 3). *Lycium* seeds from *Falco* pellets had the thinnest coats of all treatments; these were significantly different from control seeds (16% of reduction; ANOVA *I*-test, $F = 6.67$, d.f. = 3, 53, $P = 0.001$). The greatest changes in seed coat thickness, however, were observed in *Rubia*, especially for seeds from *Falco* pellets (43% of reduction with respect to control seeds; $F = 127.29$, d.f. = 3, 56, $P < 0.001$). Unfortunately, the small sample size for *Asparagus* did not allow any statistical analysis, although the seeds of this species showed the smallest percentage of coat reduction (Table 3). Seed hardness in *Asparagus* was unaffected by ingestion by any disperser ($\chi^2 = 3.17$, d.f. = 3, $P = 0.36$). Despite the fact that it was impossible to measure the hardness of *Lycium* and *Rubia* seeds, due to flexibility restrictions (see Methods), *Lycium* seeds clearly show the softest consistency whereas *Rubia* seeds showed an apparent intermediate robustness between those of *Lycium* and *Asparagus*.

SEED VIABILITY AND GERMINATION

The viability of control seeds was high (over 90%) in all species (Fig. 1). For *Lycium* and *Rubia*, no significant differences were observed among seeds from control plants, *Gallotia* droppings and *Lanius* pellets. However, when *Falco* was involved as a secondary seed agent, viability was significantly reduced (43.6% in *Lycium* and 29.3% in *Rubia*) with respect to control seeds

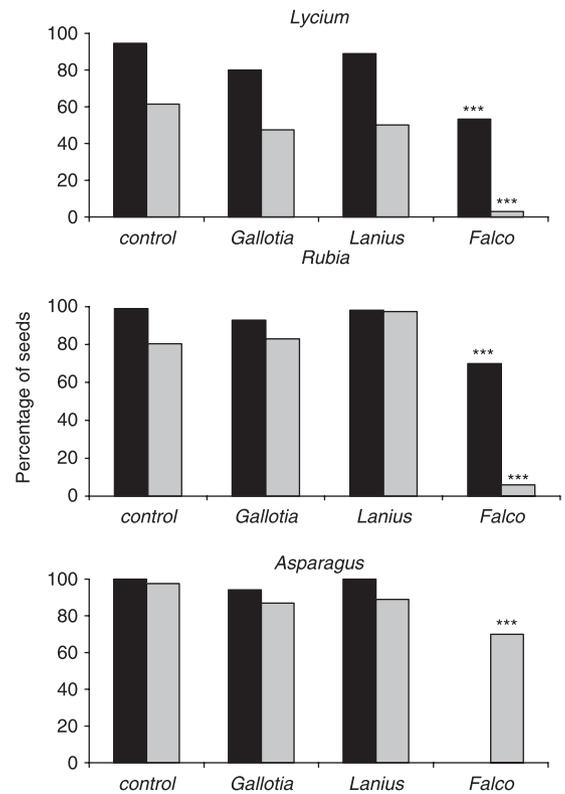


Fig. 1. Viability and germination of the different treatments of the seeds of *Lycium*, *Rubia* and *Asparagus* in Malpais de La Corona (Lanzarote, Canaries). Black bar: viability, grey bar: germination. Viability of *Asparagus* seeds in *Falco* pellets was not analysed due to the rarity of this interaction. Significant differences, *** $P < 0.001$.

(Likelihood ratio test, *Lycium*: $G = 20.32$, d.f. = 1, $P < 0.001$; *Rubia*: $G = 13.82$, d.f. = 1, $P < 0.001$) and with respect to those seeds extracted from *Lanius* pellets (*Lycium*: $G = 13.38$, d.f. = 1, $P < 0.001$; *Rubia*:

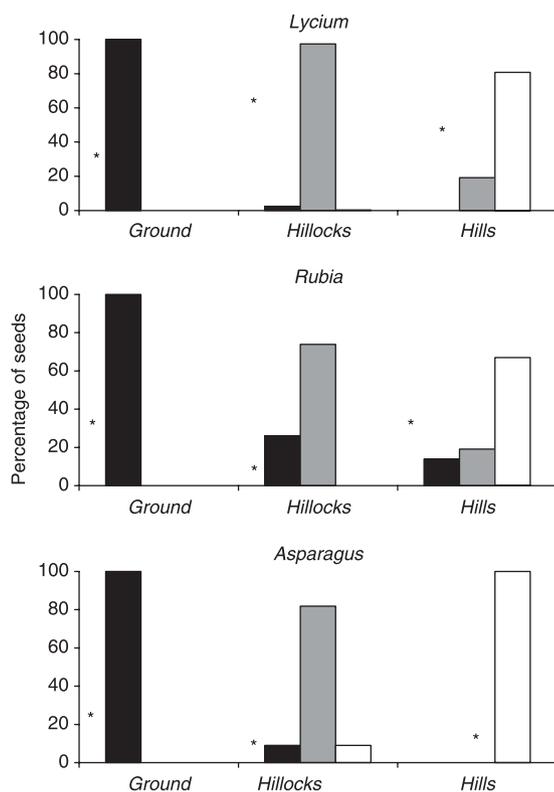


Fig. 2. Seed distribution pattern (spring 2004 and 2005 combined) of *Lycium*, *Rubia* and *Asparagus* across the three main microhabitats considered, produced by the disperser agents in Malpais de La Corona (Lanzarote, Canaries). Black bars: *Gallotia*; grey bars: *Lanius*; white bars: *Falco*. Asterisks represent the percentage of occurrence of the different plant species in the squares (0.25 m²) sampled in the three main microhabitats.

$G = 14.92$, d.f. = 1, $P < 0.001$). No significant effect of gut treatment on viability was observed in the case of *Asparagus* seeds.

The percentage of seeds that germinated for the three plant species was similar in control, *Gallotia* and *Lanius* treatments (Fig. 1). However, germination decreased for all species when seeds were consumed by *Falco* (Likelihood ratio tests, $P < 0.001$ for all comparisons). In the case of *Lycium* and *Rubia* seeds, the reduction was very large (95.1% and 92.5%, respectively), compared to control seeds, while it was moderate (28.2%) in *Asparagus* seeds.

The speed at which seeds germinated differed among the three plant species studied: *Lycium* seeds from three treatments (control, *Gallotia* and *Lanius*) germinated earlier than those from *Falco* pellets (Kolmogorov–Smirnov tests, $P < 0.001$). For *Rubia*, seeds from *Lanius* were the fastest to germinate compared to the remaining treatments ($P < 0.001$), while control and *Gallotia* (statistically similar) seeds germinated sooner than those from *Falco*. Lastly, in the sole case of *Asparagus* seeds where sample size was suitable for comparison purposes, no differences were observed between control seeds and those from *Gallotia* droppings ($Z = 1.06$, $P = 0.20$).

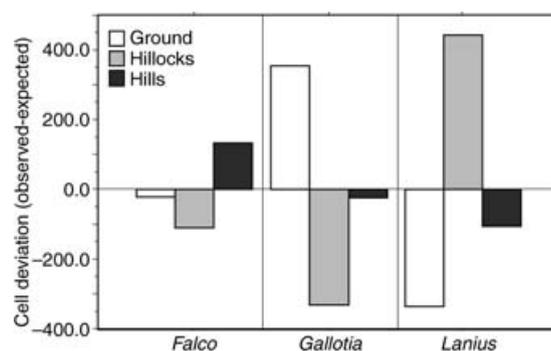


Fig. 3. Residuals of a contingency table analysis (observed cell frequency minus the expected cell frequency of dispersed seed) including disperser species and microhabitat. Bars depict the residual frequencies of dispersed seeds and their significance, indicating microhabitats favoured (positive significant residuals) or avoided (negative significant residuals) by each disperser. All values, *** $P < 0.001$.

SEED DISTRIBUTION, FLESHY FRUIT PLANT DISTRIBUTION AND VERTEBRATE MOVEMENTS

Spatial patterns of seed deposition produced by the vertebrates involved were clearly different: *Gallotia* deposited most seeds in the open ground microhabitat, *Lanius* in the hillocks and *Falco* in the hills (Fig. 2). The differences among dispersers were significant for all three species: *Lycium* ($G = 1474.4$, d.f. = 4, $P < 0.001$), *Rubia* ($G = 912.5$, d.f. = 4, $P < 0.001$) and *Asparagus* ($G = 14.6$, d.f. = 4, $P = 0.006$). Furthermore, microhabitats differed strongly in the proportions of seed contributed by the different dispersal agents ($G = 3059.5$, d.f. = 4, $P < 0.001$) (Fig. 3). All seeds arriving in the open ground habitat were dispersed by *Gallotia*, while most seeds arriving to hillocks and hills were dispersed by both predatory birds. In addition, seeds from the three plant species differed in their distribution among habitats ($G = 785.9$, d.f. = 4, $P < 0.001$). A total of 89% of *Lycium* seeds and 73% of *Asparagus* seeds were recorded in the hillocks, while 44% and 41% of *Rubia* seeds were found in the open ground and hillocks, respectively.

Movements of three dispersers were notably different. As expected, *Falco* moved the longest distances (506.4 ± 361.2 m; range: 75–1500 m; $n = 35$ movements; $n = 22$ different individuals), followed by *Lanius* (76.0 ± 49.9 m; range: 10–250 m; $n = 34$ movements; $n = 26$ different individuals) and by *Gallotia* (1.22 m; range: 0.6–2.5 m; $n = 32$ movements; $n = 6$ different individuals; A.I. Gómez & C. Hernández, pers. comm.). The 'dispersal kernel' analysis indicated that flights carried out by *Lanius* were more frequently shorter than those performed by *Falco* (Fig. 4; $G = 64.9$, d.f. = 7, $P < 0.001$). Lastly, with regards to the seed movements documented beneath the perches of both predatory birds in the hillocks and hills located in the cultivated area of Llanos de Órzola, *Lanius* moved *Asparagus* seeds a mean of 608 ± 229 m (range: 350–1150 m;

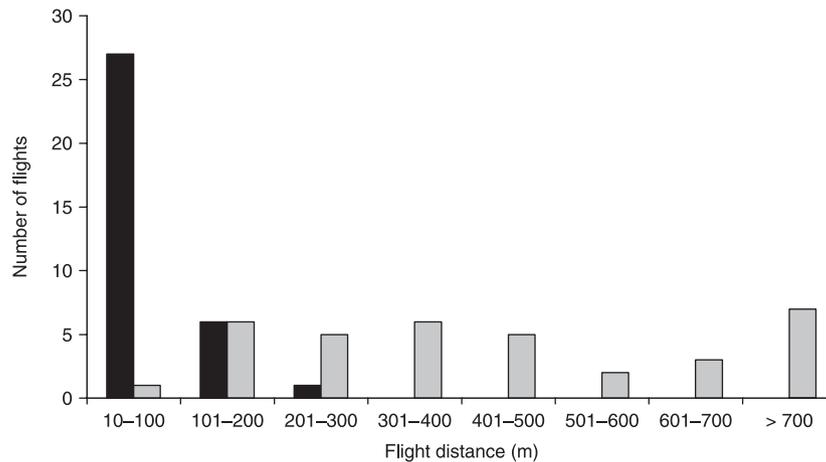


Fig. 4. 'Dispersal kernel' analysis carried out for those flights performed by both predatory bird species in Malpaís de La Corona (Lanzarote, Canary Islands). Black bars: *Lanius*; grey bars: *Falco*.

$n = 10$), while the mean movement of seeds by *Falco* was 775 ± 434 m (range: 275–1350 m; $n = 6$). These distances did not differ significantly ($U = 24.0$, $P = 0.51$).

Discussion

THE DISPERSAL SYSTEMS

Frugivorous lizards from the endemic genus *Gallotia* are frequently captured by predatory birds throughout the Canarian archipelago (Nogales *et al.* 2002). These predatory events are interesting in that whereas a double interaction of birds involved in the diplochory by endozoochory has already been described in continental environments (Ridley 1930; Damstra 1986; Hall 1987; Dean & Milton 1988), our three insular seed-dispersal systems included an endemic frugivorous lizard as the main primary disperser. The diverse physiological treatments on seeds carried out by birds and reptiles could have different effects on seed viability and germination of plants (Traveset 1998). Lizards are often abundant in insular environments, and the Canary Islands are not an exception. In addition, they are frequently involved in ecological processes such as frugivory and seed dispersal (Olesen & Valido 2003 and references therein). The diplochorous system studied here, based on predation on lizards by two predatory birds (a shrike and a kestrel), has also been observed on other islands of the Canaries. The shrike has been reported as a secondary seed dispersal agent in those islands where it is present (Lanzarote, Fuerteventura, Gran Canaria and Tenerife) and the kestrel in practically all the islands (Padilla *et al.* 2005; D.P. Padilla & M. Nogales, unpubl. data). For this reason, at least in the Canary Islands, diplochory by double endozoochory is a relatively common seed dispersal process. Further, this supports the idea of Vander Wall & Longland (2004), who argue that diplochory is a common means of seed dispersal in both temperate and tropical communities.

SEED MOVEMENTS

The greater number of *Lycium* seeds dispersed than the other two species is at least partly accounted for by the fact that their berries produce a mean of 7.43 ± 3 seeds (Nogales *et al.* 2002), many more than *Rubia* (1.4 ± 0.3 ; Nogales *et al.* 1999) and *Asparagus* (1.0 ± 0 ; $n = 40$ fruits; pers. obs.). Therefore, the ingestion of *Lycium* fruits by the primary dispersers (lizards) strongly conditions that individual lizard prey will be highly loaded with such seeds. Furthermore, the fruiting period of *Lycium* extends from January to May, compared to a shorter fruiting period for the other two plant species. Given the similar chemical composition of the pulp of the three fruits consumed by lizards (unpubl. data), consumption might be mainly determined by seasonal availability of fruits.

The number of seeds transported by each dispersal agent is one of the most important factors in seed dispersal effectiveness (Schupp 1993). In this study, the interaction *Gallotia* \times *Lanius* carried more than 65% of the total seeds sampled, followed in importance by those seeds directly dispersed by *Gallotia* and the interaction *Gallotia* \times *Falco*. However, although defecation and regurgitation rates range between one and two droppings/pellets per day for the three dispersers (Duke *et al.* 1976; Olsson 1985; A. Valido & M. Nogales, unpubl. data), a higher number of seeds are probably transported by lizards due to their great abundance in insular systems (Olesen & Valido 2003).

SEED TRAITS AFTER GUT TREATMENT

External damage to seeds appeared low for the different plant species and treatments. These data are consistent with earlier studies on secondary seed dispersal systems where a double digestion process occurs (Nogales *et al.* 1998, 2002).

The decreased seed coat thickness following gut passage has already been documented (Traveset *et al.*

2001; Nogales *et al.* 2005), although the effect of such changes on germination was largely unknown. Seed hardness might play a more important role in seed germination than seed coat thickness, as the hardest seeds (*Asparagus*) were the only ones that survived after the gut treatment of *Falco*. The importance of seed hardness in seed survival has been previously pointed out in legume seeds passing through the digestive tract of cattle (Gardener *et al.* 1993) and in seeds from shrubs such as *Sorbus* (Paulsen *et al.* 2006), *Phillyrea* and *Myrtus* after passing through birds' guts (Traveset *et al.*, in press). Thicker seed coats were not found to be more abraded than thinner coats and other seed coat variables need to be examined to determine which seed traits affect survival and changes in viability and germination after ingestion by dispersers.

SEED VIABILITY AND GERMINATION

Gallotia and *Lanius* did not negatively affect the viability and germination of seeds, although *Falco* clearly did. This effect is important for viability and has profound consequences for germination in the case of *Lycium* and *Rubia* seeds. Therefore, these data suggests that *Falco* is a less effective secondary seed disperser than *Lanius*. These results coincide with previous findings by Nogales *et al.* (1998, 2002) in the case of *Lycium* plants on Alegranza Island. The enzymatic action of the diurnal raptor, which is clearly stronger than in many other types of birds (Duke *et al.* 1976; Brown *et al.* 1993; Stuart & Stuart 1994), probably has a negative influence on seed survival. Furthermore, another effect that influences seed fate is gut passage time, which in the case of *Falco* (12–23.5 h; Balgooyen 1971; Duke *et al.* 1976; Yalden & Yalden 1985) is much longer than in *Lanius* (45–55 min; Olsson 1985). However, in the case of *Asparagus*, the percentage of seed germination was still high (70%), which demonstrates that this raptor can function as an appropriate secondary disperser for some plants. Seed dormancy was not observed in the case of *Asparagus*, while it was slight in *Rubia* and scarcely more important in *Lycium* seeds.

Speed of germination in the three species was clearly affected by dispersers. This differential effect on seed emergence caused by lizards and birds might be important in geographical zones where rainfall is unpredictable (e.g. the Mediterranean zone) and where the probability that seedlings can recruit successfully and for a longer period is raised (Izhaki & Safriel 1990; Traveset *et al.* 2001).

In the context of LDD events (e.g. large movements between islands), and according to the data presented in this contribution on seed coat thickness, hardness, viability and germination, *Lanius* seems to be an effective disperser for a higher number of plants than *Falco*. However, this raptor – with clearly longer gut retention times and power of flight – would be an effective disperser in the case of some plants such as *Asparagus*.

SEED SPATIAL PATTERN PRODUCED BY DISPERSERS AND PLANT DISTRIBUTION

The three plant species were present in all three main microhabitats identified (open ground, hillocks and hills) which suggests that the origin of these plants could be associated with differential habitat use by the distinct dispersers. This is supported by the fact that seed distribution produced by the primary and secondary dispersers was markedly different. *Gallotia* lizards are more frequently found in open ground (flat rocky places with shrubby vegetation), while *Lanius* use the small hillocks as perches and *Falco* the hills. The first two species function as dispersers for the different species and are probably responsible for their presence in both microhabitats. In the case of the hills, *Falco* is the sole agent responsible for the transport of all *Asparagus* seeds recorded. Taking into account the damage caused by this raptor to seeds of *Lycium* and *Rubia*, the presence of *Asparagus* could be associated with its ecological effect.

These results indicate that *Lanius* is a more frequent LDD vector than *Falco* and that this shrike transports most seeds to a particular microhabitat, the hillocks. However, *Falco* more frequently carried out longer flights than *Lanius* and this could be important for LDD events of those seeds that are able to resist the strong gut treatment. Furthermore, it is convenient to consider that *Falco* may dismember its prey before ingestion. Hence, an unknown percentage of seeds remain inside the digestive tract of the primary disperser (the lizard), having undergone only a single digestive process and remaining in a favourable state of viability (D.P. Padilla & M. Nogales, unpubl. data). Thus, *Falco* might also be involved in LDD events of *Lycium* and *Rubia* to the hills, despite the negative effect recorded on those seeds found in its pellets.

SEED DISPERSAL OF FLESHY-FRUITED PLANTS IN RECENT INSULAR VOLCANIC AREAS

In recent volcanic zones, lava flows can create a matrix of young and old substrates. However, massive eruptions can cover large areas with young volcanic materials, creating ideal frameworks to study animal and plant colonization. Furthermore, oceanic islands are simple systems compared to continental ones, and therefore their functioning can be better understood, as occurs with the seed dispersal system of the three plants studied here.

Although a large proportion of seeds from fleshy fruits are dispersed in oceanic subtropical island habitats by primary dispersers, mainly lizards and small passerines (Valido & Nogales 1994; Nogales *et al.* 2005), some seeds are transported by large long-distance dispersers (principally ravens or gulls; Nogales *et al.* 1999; Nogales *et al.* 2001). However, in addition to these primary seed dispersal systems, the

ecological framework in which this study was developed permits us to assess other complex ecological phenomena, such as diplochory by double endozoochory, an ecological process which, according to all the data presented in this study, could potentially have significant consequences for long-term seed dispersal processes. Although the current paradigm on LDD suggests that these ecological processes occur rarely and are due to infrequent ecological events (Greene & Johnson 1995; Higgins & Richardson 1999; Clark *et al.* 2001), the LDD systems we are dealing with are regular and affect a great number of seeds that are being moved every year. Furthermore, these LDD systems probably accelerate plant colonization of badlands. In this regard, it is important to consider that maximum values of the minimum long-distance seed dispersal recorded for *Asparagus* in the study area was 1.15 km and 1.35 km for *Lanius* and *Falco*, respectively.

According to the gut passage time of both predatory birds, seeds remain about 45–55 min in the intestinal tract of *Lanius* (Olsson 1985) and 12–23.5 h in *Falco* (Balgooyen 1971; Duke *et al.* 1976; Yalden & Yalden 1985), and these birds reach mean cruising flight speeds of 34.0 km per h for *Lanius* (pers. obs.) and 40.9 km per h for *Falco* (Meinertzhagen 1955; Campbell & Lack 1985). Although the mean movements recorded for these birds were about 76 m and 506 m, respectively, in the hypothetical case that these birds were to fly a long distance, *Lanius* could cover about 26–31 km and *Falco* about 491–961 km before expelling the seeds, which provides insight into the potential magnitude of LDD processes in the present seed dispersal systems.

The results simultaneously obtained on the variety of ecological factors analysed in this study support the hypothesis that diplochory by double endozoochory could play a more important role in LDD events than is currently recognized, both in recent volcanic areas (lowlands) and also probably in the colonization of other islands (see Moore 1999). Furthermore, it is convenient to consider that these complex seed dispersal processes show a selective trade-off between the long gut retention time of seeds necessary to complete a LDD event, and the seed damage-cost by scarification associated with such long retention times. Our results demonstrate that while some plants may resist these ecological events, others are less likely to survive. Lastly, to have a more complete idea of the real importance of this kind of LDD system in the Canary Island flora, it will be necessary to evaluate the number of plant species that are being dispersed in the different islands by this particular multi-step process and the ecological fate of their seeds.

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