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**EFFECT OF SEED PASSAGE THROUGH BIRDS AND LIZARDS ON  
EMERGENCE RATE OF MEDITERRANEAN SPECIES: DIFFERENCES  
BETWEEN NATURAL AND CONTROLLED CONDITIONS**

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## 1 **Summary**

2

3 **1.** The ingestion of fruits by vertebrate frugivores produces great variation on seed  
4 germination responses which depend on (1) the frugivorous species, (2) several factors  
5 intrinsic to the plant species and (3) the conditions under which germination tests are  
6 conducted. Most studies on this topic have used only one or a few disperser species, and  
7 have been performed under controlled conditions.

8 **2.** The main goals of this study were to determine (1) the effect that birds (*Turdus*  
9 *merula*) and lizards (*Podarcis pityusensis*) have on seed emergence patterns of a group  
10 of common Mediterranean plants, and (2) whether such patterns differ between field  
11 and common garden conditions. For over two years, we compared seed emergence time,  
12 with those of controls (pulp-removed seeds).

13 **3.** Emergence patterns were inconsistent for most plant species when comparing field  
14 vs. common garden conditions. In some cases, results were even contradictory: for  
15 instance, lizards accelerated the emergence time of *Rubus* seeds in the field, but delayed  
16 it in the experimental garden; likewise, *Rubia* seeds ingested by blackbirds emerged  
17 more slowly than controls in the field but faster than those in the garden. The two  
18 frugivorous species had also inconsistent effects on the same plant species.

19 **4.** Significant changes in seed weight after passage through the animals' digestive tracts  
20 were observed in most species. However, seed weight did not explain differences in  
21 seed emergence patterns between ingested and non-ingested seeds.

22 **5.** This study demonstrates the great heterogeneity in seed emergence responses of the  
23 different plant species to ingestion by different types of frugivores, and to the different  
24 experimental conditions, and thus caution is needed when making generalizations from  
25 this type of studies.

## 1 **Introduction**

2  
3  
4 Despite it has been assumed for a long time that one of the advantages of endozoochory  
5 is germination enhancement –i.e., that seeds ingested by frugivores germinate in greater  
6 proportions or faster than non-ingested ones- (Traveset & Verdú 2002, and references  
7 therein), an increasing number of studies have shown that such enhancement is far from  
8 universal and that a variety of factors, both biotic and abiotic, may affect the outcome of  
9 seed treatment in the frugivores' digestive tracts (e.g. Traveset *et al.* 2001; Santamaría  
10 *et al.* 2003; Espinar *et al.* 2004; Verdú & Traveset in press). This is of crucial  
11 importance to determine the quality of a particular disperser for a plant.

12         The conditions under which germination tests are performed are known to  
13 influence germination success (e.g. Bustamante *et al.* 1993, Figueiredo & Perin 1995;  
14 Traveset *et al.* 2001). It is thus important that seed responses to dispersers' gut  
15 treatment are examined in the field, as it is in natural environments where we can test if  
16 a germination enhancement is adaptive or not. So far, most reported information comes  
17 from studies carried out under controlled (and thus favourable) circumstances, usually  
18 in the laboratory (see review in Traveset & Verdú 2002), yet this may often obscure  
19 significant differences between treatments (Herrera 2000, Traveset *et al.* 2001).

20         On the other hand, most studies which experimentally test the effect of gut  
21 passage on seed germination have been performed with only one or a few dispersers of  
22 all those available for the plant. Birds and non-flying mammals are the frugivorous taxa  
23 for which most data are available, as they are important dispersers for a great variety of  
24 plants (see review in Traveset 1998); in contrast, little is known about the effect of  
25 reptiles (but see Liu *et al.* 2004, and references therein), despite they are also effective  
26 dispersers in some ecosystems, mainly in islands (Olesen & Valido 2003). Studies that  
27 compare the effect of different groups of dispersers on germination are scarce and have  
28 shown either consistent (e.g. Krefting & Roe 1949, Lieberman & Lieberman 1986,

1 Mandujano *et al.* 1994, Traveset & Willson 1997) or inconsistent results (e.g.  
2 Lieberman & Lieberman 1986, Figueiredo & Perin 1995, Engel 1997, Nogales *et al.*  
3 1998, Nogales *et al.*, submitted). The latter are often attributed to the different retention  
4 times in the animals' guts (e.g., Izhaki & Safriel 1990, Barnea *et al.* 1991, Murphy *et al.*  
5 1993, but see Barnea *et al.* 1990, Traveset *et al.* 2000, 2001), although other factors  
6 such as type of food ingested along with the fruits (with variable water content, pH,  
7 proportion of plant material, etc.) are likely to influence the level of mechanical or  
8 chemical scarification of the ingested seeds (e.g., Traveset 1998, Traveset *et al.* 2001,  
9 Figuerola *et al.* 2002).

10         In the present study, we chose fleshy-fruited plant species common in the  
11 Mediterranean Basin, plus one endemic to the Balearic Islands, to examine the effect of  
12 seed passage through frugivores' digestive tracts on emergence rate (speed at which  
13 seeds emerge), simultaneously examining both in the field and in an experimental  
14 garden during two years. Hereafter, we will indiscriminately use the terms germination  
15 and emergence time, even though what we did measure was the time at which the  
16 seedling emerges. For five of the species known to be dispersed both by birds and  
17 lizards (Sáez & Traveset 1995) we compared the effect of these two types of dispersers.  
18 Given the much longer time seeds are retained within the digestive tract of lizards (2-4  
19 days) compared to birds (usually 20-30 min.), we hypothesized that the former were  
20 more likely to have a significant effect on germination patterns. We further predicted  
21 that differences in such patterns would not be similarly detected in the field than in the  
22 experimental garden, mainly due to the more favourable conditions -mainly temperature  
23 and humidity- in the latter.

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## 1 **Material and methods**

2

### 3 **STUDY PLANT SPECIES**

4

5 From August 2000 to January 2001, we collected fruits from 11 fleshy-fruited species  
6 common in the western Mediterranean flora, plus one endemic species from the  
7 Balearic Islands. Fruits of each species were obtained from a minimum of 10  
8 individuals, simultaneously and from the same site to avoid any effect of seed age or  
9 source on germination. The species were: *Crataegus monogyna* (Rosaceae), *Ephedra*  
10 *fragilis* (Ephedraceae), *Juniperus phoenicea* (Cupressaceae), *Osyris alba* (Santalaceae),  
11 *Pistacia lentiscus* (Anarcadiaceae), *Rhamnus alaternus* and *R. ludovici-salvatoris*  
12 (Rhamnaceae; the latter endemic to the Balearic Islands), *Rubus ulmifolius* (Rosaceae),  
13 *Rubia peregrina* (Rubiaceae), *Solanum luteum* and *S. nigrum* (Solanaceae), and *Smilax*  
14 *aspera* (Smilacaceae). Hereafter we will refer to them by their generic names only, except  
15 for the two *Rhamnus* and two *Solanum* species. The main fruit characteristics of all  
16 these species are given in Table 1.

17

### 18 **FRUGIVOROUS SPECIES**

19

20 The bird species chosen for the study was the blackbird (*Turdus merula*; Turdidae), one  
21 of the most important dispersers of fleshy-fruited plants in the Balearic Islands; it is  
22 sedentary and relatively abundant in these islands. We captured three individuals during  
23 summer 2000 and two during summer 2001 with mist-nets at several sites from an  
24 orchard site (this last year, this species was especially rare due to a fungus infection that  
25 killed many individuals). Captured birds were kept in an indoor aviary, and housed  
26 separately in cages (size: 60 x 60 x 60 cm) until the end of the experiment, when they  
27 were released at their sites of origin. They were maintained on a mixed diet of beetle

1 larvae (*Tenebrio molitor*), commercial food for insectivorous birds and wild fleshy  
2 fruits. Blackbirds swallowed entire fruits of all tested species.

3         The lizard species used for the experiments was *Podarcis pityusensis*  
4 (Lacertidae), endemic to the Pithyusic Islands (Balearic Archipelago, Western  
5 Mediterranean). This lizard (snout-vent-length: c. 10 cm) is omnivorous, including an  
6 important fraction of plant material in its diet (Riera, unpub. data). Twenty individuals  
7 were captured during the spring 2000 in Formentera Island and transported to our lab in  
8 Esporles (Mallorca). They were kept in two indoor terraria (size: 30 x 70 x 40 cm) and  
9 were fed with a diet consisting of beetle larvae, tomatoes, and commercial cat food.

10         During the frugivore ingestion experiments, fresh fruits of each species were  
11 collected as they became available in the field. They were given to each individual bird  
12 or lizard with an interval period of 24 h and 36 h, respectively, to avoid any seed-  
13 mixing effects.

14

## 15 SEED GERMINATION EXPERIMENTS

16

17 Seeds from bird droppings were collected from cages while still fresh and cleaned by  
18 means of towel paper. A similar sample of seeds was extracted and cleaned from fruits  
19 which were randomly chosen from the same source used for bird and lizard feeding. All  
20 seeds were individually weighted to the nearest 0.1 mg in order to determine if passage  
21 through the digestive tract of birds modified seed weight, as found in other studies  
22 (Traveset *et al.* 2001; Paulsen & Högstedt 2002). Seeds from lizard droppings were  
23 collected from the terraria and also cleaned, but they were not weighted as they were  
24 not considered to be fresh enough at the time of collection. All seeds were kept in paper  
25 bags, in dark and dry conditions, until the date of sowing. A total of 200 seeds of each  
26 species were used as controls (with the exception of *Osyris* and *Rubus*, with only 195

1 and 197 seeds, respectively). We also obtained 200 seeds of each species ingested by  
2 either birds or lizards, except in the case of *Rubia*, for which we could obtain only 100  
3 seeds of the lizard treatment. Five of the species (*Crataegus*, *Juniperus*, *Osyris*, *Pistacia*  
4 and *Smilax*) were fed only to birds as they were too large to be swallowed for lizards.  
5 For *Ephedra* and *R. alaternus* we could only gather a limited amount of fruits and we  
6 thus decided to test these species on a single disperser (birds for *Ephedra* and lizards for  
7 *R. alaternus*); the effect of ingestion by *Turdus merula* on germination patterns of *R.*  
8 *alaternus* had already been tested by Barnea *et al.* (1991). All seeds selected for sowing  
9 were apparently viable; aborted seeds (usually of a smaller size and/or different colour)  
10 were previously discarded.

11

#### 12 *In the field*

13 The area used to test seed germination was a Mediterranean scrubland located about one  
14 km from our laboratory, where the experimental garden is located. Seeds of *Crataegus*,  
15 *Osyris* and the two *Solanum* species were planted near a temporary stream, as these  
16 species are usually found in relatively humid conditions, whereas the rest of species  
17 were sown in an open site where shrubs of *Pistacia lentiscus*, *Olea europea*, *Rhamnus*  
18 *alaternus*, *Rubus ulmifolius*, *Ceratonia siliqua* and *Smilax aspera* are relatively  
19 abundant. At each location (open site and near stream), we divided the area in five  
20 quadrats (stations, hereafter) and planted the seeds of all species and treatments in them.  
21 Before planting seeds, superficial land and weeds were removed in order to prevent  
22 germinations from the seed bank. In each station we planted groups of 20 seeds of each  
23 treatment and species, equally spaced and separated 5 cm from each other. Seeds were  
24 planted at a depth of 0.5-1 cm, and each group was protected with a cage (30 x 20 x 2.5  
25 cm) made of wire mesh to prevent rodent predation. Stations were separated by at least  
26 5 m from each other. Feeding experiments were performed at different times depending

1 upon fruit availability in the field, and thus seed sowing was not simultaneous for all  
2 species either: seeds of *Rubus*, *Rubia*, *Smilax*, *Osyris*, *Crataegus* were planted on 1-5<sup>th</sup>  
3 December 2000, *Pistacia* on 9<sup>th</sup> February 2001, *Juniperus* and the two *Solanum* species  
4 on 7<sup>th</sup> March 2001 and *Ephedra* and the two *Rhamnus* species on 28<sup>th</sup> November 2001.  
5 Germination and seedling survival was monitored weekly or every two weeks,  
6 depending of seed germination rate, until 27<sup>th</sup> December 2002 when no new seedlings  
7 had germinated for 7 months.

8

### 9 *In the experimental garden*

10 Simultaneously to the plantation in the field, seeds of each species and treatment were  
11 sown in the experimental garden. They were individually planted in trays of 60 pots (4 x  
12 4 cm) each, filled with horticultural mixture, at a depth of about 0.5 cm. Trays were  
13 periodically watered to ensure permanent humidity and seed germination was monitored  
14 every 7-10 days until 9<sup>th</sup> September 2002, when no seedlings had germinated for about  
15 five months.

16

### 17 DATA ANALYSIS

18 Differences in seed weight between treatments were tested by means of General  
19 Linear Model, after logarithmically transforming the data to reach normality. These  
20 analyses were performed using the GENMOD procedure of SAS 8.02 package for  
21 Windows (SAS Institute, 2001).

22 The effect of seed ingestion on seed emergence time was tested separately for  
23 each plant species using failure-time analysis. A Cox proportional hazard regression  
24 model was fitted to the number of days between sowing and emergence for each seed.  
25 Using the parametric accelerated failure-time model, results were identical. Non-  
26 germinated seeds were modelled as right non-censored data points due to the



1 uncertainty that they could eventually germinate after the study period; for such seeds,  
2 we thus considered the failure time as the number of days until the end of the  
3 experiment. Actually, species such as *Crataegus* and *Juniperus* are known to have  
4 physiological dormancy (Baskin & Baskin 1998); *Smilax* is also likely to have such  
5 dormancy, given the low germination found in this study and elsewhere (Izhaki &  
6 Safriel 1990; Traveset *et al.* 2001). Other species like the two *Solanum*, *Rubus*, *Rubia*  
7 and *R. alaternus* might not have seed dormancy; however, we decided to use a  
8 consistent model for all species used in the study. For the field data, station was added  
9 as a random or ‘frailty’ effect in the model as all treatments were applied to each  
10 station. Ties were estimated using the efron method, using the program S-Plus 2000  
11 (Mathsoft 1999).

12

## 13 **Results**

14

### 15 GERMINATION IN THE FIELD

16

17 Out of the 12 tested species, we found that seed ingestion by dispersers (either birds,  
18 lizards or both) affected seed germination rate in four species: *Rubus*, *Rubia*, *R.*

19 *ludovici-salvatoris*, and *S. nigrum* (Fig. 1 & 2). No seeds of *Pistacia* germinated in the  
20 field during the study period, and thus this species was excluded from the analysis.

21 Differences between treatments were only marginally significant in the case of *Rubus*  
22 (between lizards and control seeds) and in the case of *Rubia* and *R. ludovici-salvatoris*

23 (between blackbirds and control seeds): blackbirds appeared to marginally delay seed

24 germination in *R. ludovici-salvatoris* ( $\chi^2 = 3.47$ ,  $df = 1$ ,  $P = 0.063$ ) and *Rubia* ( $\chi^2 = 3.80$ ,

25  $df = 1$ ,  $P = 0.051$ ). However, lizards significantly accelerated germination in *Rubia* ( $\chi^2 =$

26  $5.87$ ,  $df = 1$ ,  $P = 0.015$ ) and *Rubus* ( $\chi^2 = 3.03$ ,  $df = 1$ ,  $P = 0.082$ ). The two *Solanum*

1 species responded differently to ingestion by dispersers: while seeds of *S. nigrum*  
2 ingested by either disperser significantly advanced germination (blackbirds:  $\chi^2 = 4.97$ ,  
3  $df = 1$ ,  $P = 0.026$ ; lizards:  $\chi^2 = 4.20$ ,  $df = 1$ ,  $P = 0.040$ ), in the case of *S. luteum*, no  
4 difference was observed between ingested and non-ingested seeds (Fig. 2).

5

## 6 GERMINATION IN THE EXPERIMENTAL GARDEN

7

8 Results obtained in the garden were rather different from those in the field (Fig. 1 & 2).  
9 Under controlled conditions, we detected a significant effect of seed passage through  
10 frugivores on seed germination rate in *Rubus*, *Rubia*, *S. luteum*, and *Pistacia*. In contrast  
11 to field results, *Rubus* seeds ingested by either birds or lizards delayed germination  
12 compared to control seeds (blackbirds:  $\chi^2 = 6.97$ ,  $df = 1$ ,  $P = 0.009$ ; *Podarcis*:  $\chi^2 = 6.86$ ,  
13  $df = 1$ ,  $P = 0.008$ ; Fig. 2). For *Pistacia*, despite seeds ingested by blackbirds showed a  
14 reduction of germination time, only 8 control seeds germinated in front of one from the  
15 bird treatment (Fig. 1), and thus the statistical difference observed has not much  
16 biological sense. In contrast to field results, the seeds of *Rubia* and *S. luteum* ingested  
17 by blackbirds germinated faster than control seeds (*Rubia*:  $\chi^2 = 4.55$ ,  $df = 1$ ,  $P = 0.023$ ;  
18 *S. luteum*:  $\chi^2 = 4.17$ ,  $df = 1$ ,  $P = 0.037$ ; Fig. 2), but ingestion by lizards had no  
19 significant effect (*Rubia*:  $\chi^2 = 0.55$ ,  $df = 1$ ,  $P = 0.780$ ; *S. luteum*:  $\chi^2 = 0.06$ ,  $df = 1$ ,  $P =$   
20 0.980).

21

## 22 CHANGES IN SEED WEIGHT AFTER INGESTION

23

24 Seed passage through the digestive tract of blackbirds influenced seed weight in most  
25 plant species, although not consistently in the same direction. Most species suffered a  
26 slight loss of seed weight after ingestion by blackbirds, whereas defecated seeds of three  
27 species (*Ephedra*, *Rubus* and *Smilax*) were heavier after being ingested (Table 2). Such

1 differences in seed weight were not related to seed size; the correlation between  
2 maximum mean diameter and proportional mean weight change between ingested and  
3 non ingested seeds was not significant ( $r^2 = 0.042$ ,  $n = 10$  species,  $P = 0.561$ ). All fruits  
4 we gave to birds in the cages were eaten, and thus the possibility that birds had selected  
5 larger or smaller fruits than controls is also discarded.

6

## 7 **Discussion**

8

9 Seed passage through the digestive tract of frugivores has long been found to affect the  
10 germinability and/or the germination rate in many plant species, which together with the  
11 seed shadow and the quantity of 'intact' dispersed seeds are essential factors that  
12 determine plant reproductive and regeneration success (e.g. Schupp 1993). However,  
13 only a low fraction of studies have performed germination tests in the field and they  
14 have used either one or a few dispersers (Traveset & Verdú 2002). There are also a few  
15 studies showing that experimental conditions under which tests are carried out may lead  
16 to different responses in germination, and that such differences do not consistently go in  
17 the same direction. One group of studies shows that differences in seed germination  
18 between treatments are usually magnified under harsh (field) conditions; for example,  
19 Herrera (2000) only detected germination differences between seeds from two  
20 pollination regimes when planted in the field, but not in the greenhouse. Similarly,  
21 Traveset *et al.* (2001) detected a positive effect of bird ingestion on seed germination in  
22 outdoor conditions but not in a growth chamber or in a greenhouse. Another group of  
23 studies shows the opposite direction, with a greater effect in laboratory compared to  
24 field experiments (Bustamante *et al.*, 1992, 1993; Figueiredo & Perin 1995; Yagihashi  
25 *et al.* 1998). Finally, a third group of studies finds similar results between the two  
26 conditions (Figueiredo & Perin 1995; Figueiredo & Longatti 1997). The present study

1 further confirms, with a larger number of species, that a species may show germination  
2 enhancement/inhibition in the field but not in controlled conditions (in the laboratory or  
3 greenhouse) and vice versa, that a significant effect of ingestion detected in controlled  
4 conditions may be screened-off in the field. For *Rubia*, *Rubus* and *Solanum*, results  
5 were inconsistent and showed an inverse trend depending on seed germination  
6 conditions (Fig. 2). This demonstrates the caution needed when interpreting germination  
7 experiments that aim to evaluate the influence of frugivores on the quality of dispersal  
8 they provide to plants. Our suggestion is that future studies examining the quality of  
9 dispersal only in laboratory or garden conditions should also be carried out in the field,  
10 in conditions as natural as possible to the particular plant. However, under field  
11 conditions, results may also depend on environmental and temporal stochasticity.

12         The present study also evidences the inconsistencies in germination responses  
13 depending upon frugivore species. The effect of blackbirds and lizards on germination  
14 rate was consistently significant only for *S. nigrum* in the field (germination was  
15 accelerated in the two treatments compared to controls) and for *Rubus* in the garden  
16 (seeds ingested by either frugivore germinated more slowly than controls). A previous  
17 study on *S. nigrum* that tested the effect of blackbirds and Sardinian warblers (*Sylvia*  
18 *melanocephala*) on germination in a common garden (Mas & Traveset 1999) had shown  
19 a similar non-significant effect of the two bird species. Similar results to these (and also  
20 including a third bird species, *Pycnonotus xanthopygos*) had been found also by Barnea  
21 *et al.* (1990), although in that case the test was done in laboratory conditions. In both of  
22 these previous studies, a significantly positive effect was found for *S. luteum*, which is  
23 also consistent with the effect of blackbirds we found in the experimental garden in the  
24 present study. In another previous study, seeds of *Rubia* ingested by blackbirds had  
25 shown to germinate more slowly than if ingested by Sardinian warblers (Traveset *et al.*  
26 2001); in such study, *Rubus* seeds passed through warblers germinated more slowly

1 than those passed through blackbirds, although neither frugivore affected germinability.  
2 One possible explanation for the contrasting results between studies may be that seeds  
3 come from different populations, and it is thus possible that they differ in traits related  
4 to germination, such as seed coat structure, coat thickness, or seed size. The different  
5 source of seeds might be also responsible for the contrasting results found for *Osyris*  
6 (that previous study had shown an important germination enhancement in blackbird-  
7 ingested seeds compared to controls). These inconsistencies have been reported in a  
8 variety of species (e.g. Lombardi & Motta 1995; Nogales *et al.* 1999; Nogales *et al.*  
9 2001). Even seeds of the same population but of different age have given different  
10 germination responses to the same treatment (see references in Traveset 1998).

11 Differences among frugivores have often been attributed to different retention  
12 time in the guts (e.g., Barnea *et al.* 1991, Murphy *et al.* 1993, but see Barnea *et al.*  
13 1990; Traveset *et al.* 2001). Gut passage time reported for passerine birds usually range  
14 between 20 and 60 min (see review in Traveset 1998) whereas for lizards it usually  
15 varies from 32 h to 96 h (Santamaría, unpubl. data). However, our results do not  
16 evidence any consistent effect of retention time on germination responses, as all  
17 possibilities of enhancement, inhibition or neutral effect of ingestion by lizards relative  
18 to the effect of blackbirds were found. This implies that other factors, probably  
19 interacting with seed retention time, are also important determining germination speed  
20 and success. Significant effects of ingestion on germination have often been attributed  
21 to the degree of seed coat scarification, associated to morphological and physiological  
22 characteristics of the frugivores' digestive tracts (Jordano 1992; Traveset 1998). In  
23 addition, differences in the chemical composition of food ingested along with seeds can  
24 produce great differences in both seed retention time (e.g. Murray *et al.* 1994; Witmer  
25 1996; Charalambidou *et al.*, unpublished) and in mechanical or chemical abrasion of the

1 ingested seeds (Clench & Mathias 1992) with direct consequences on germination  
2 behaviour.

3         Seeds of most plants species tended to lose weight after passing through  
4 blackbirds' digestive tract, a result consistent with that of Traveset *et al.* (2001). The  
5 outcome probably depends on seed coat structure, not examined in this study.

6 Interestingly, results for the common species (*Rubus*, *Rubia* and *Osyris*) in that previous  
7 study and the present one were consistent only for *Rubia*; in contrast, seed weight of  
8 *Rubus* and *Osyris* in Traveset *et al.* (2001) did not change or significantly decreased,  
9 respectively, after bird ingestion. These inconsistencies may again be attributed to the  
10 particular seed traits of each species and population. Moreover, even the gut structure  
11 and food composition of each individual frugivore probably affects changes in seed  
12 weight. Ongoing research that examines the effect of individual plant and frugivore  
13 differences is expected to shed more light on this issue.

14         In the recent review by Traveset & Verdú (2002), seed ingestion by birds was  
15 found to have a significantly greater positive effect on germination than either non-  
16 flying mammals or reptiles, which was mainly attributed to the shorter gut-passage time  
17 of the former. The results found in the present study actually do not follow such trend  
18 for most tested species. It is thus clear that a great heterogeneity of results exists, and  
19 that we are still far from being able to predict what are going to be the consequences for  
20 a given species, with particular seed traits, and in a particular environment. Moreover, it  
21 is important to note that even if seed treatment in frugivores' guts enhances  
22 germination, this does not necessarily imply a positive effect on performance of the  
23 future plant, more specifically, seedling fitness, growth and plant fecundity (Verdú &  
24 Traveset, in press).

25         The microhabitat where the defecated seed is deposited may determine the  
26 ultimate success of the seedling and future plant (e.g., Rey & Alcántara 2000, Traveset

1 *et al.* 2003), perhaps even more important than the effect of seed treatment in the gut  
2 itself. For this reason, to know the quality of a disperser for a given plant, we should  
3 combine the effect of ingestion on seed germination change with that of depositing the  
4 seeds in different microhabitats. A particular microhabitat may be more suitable for  
5 germination and seedling establishment than others, but in turn, may enhance  
6 germination of an ingested seed more than others. This possible effect of abiotic  
7 conditions has been recently tested with two helophytes (*Scirpus littoralis* and *S.*  
8 *maritimus*); seeds ingested by ducks show a higher germination rate than control seeds  
9 in low salinity conditions but not in high salinity ones (Espinar *et al.*, 2004). Further  
10 research on how the abiotic environment influences seed germination responses to  
11 ingestion by frugivorous species is certainly necessary in order to clarify the qualitative  
12 importance that seed dispersers have on plant reproduction.

13

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15

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1 **References**

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3 Barnea, A., Yom-Tov, Y. & Friedman, J. (1990) Differential germination of two closely  
4 related species of *Solanum* in response to bird ingestion. *Oikos* **57**, 222-228.

5 Barnea, A., Yom-Tov, Y. & Friedman, J. (1991) Does ingestion by birds affect seed  
6 germination? *Functional Ecology* **5**, 394-402.

7 Bustamante, R.O., Simonetti, J.A. & Mella, J.E. (1992) Are foxes legitimate and  
8 efficient seed dispersers? A field test. *Acta Oecologica* **13**, 203-208.

9 Bustamante, R.O., Grez, A., Simonetti, J.A., Vásquez, R.A. & Walkowiak, A.M. (1993)  
10 Antagonistic effects of frugivores on seeds of *Cryptocarya alba* (Mol.) Looser  
11 (Lauraceae): consequences on seed recruitment. *Acta Oecologica* **14**, 739-745.

12 Clench, M.H. & Mathias, J.R. (1992) Intestinal transit - how can it be delayed long  
13 enough for birds to act as long distance dispersal agents. *Auk* **109**, 933-936.

14 Engel, T.R. (1997) Seed dispersal and plant regeneration by snakes? *Ecotropica* **3**, 33-  
15 41.

16 Espinar, J.L., García, L.V., Figuerola, J., Green, A.J. & Clemente, L. (2004) Helophyte  
17 germination in a Mediterranean salt marsh: Gut-passage by ducks changes seed  
18 response to salinity. *Journal of Vegetation Science* **15**, 315-322.

19 Figueiredo, R.A. & Perin, E. (1995) Germination ecology of *Ficus luschnathiana*  
20 drupelets after bird and bat ingestion. *Acta Oecologica* **16**, 71-75.

21 Figueiredo, R.A. & Longatti, C.A. (1997). Ecological aspects of the Melastomataceae  
22 by marmosets and howler monkeys (Primates: Platyrrhini) in a semideciduous  
23 forest of southeastern Brazil. *Revue d'Ecologie* **52**, 3-8.

24 Figuerola, J, Green, A.J. & Santamaría, L. (2002) Comparative dispersal effectiveness  
25 of wigeongrass seeds by waterfowl wintering in south-west Spain: quantitative  
26 and qualitative aspects. *Journal of Ecology* **90**, 989-1001.



- 1 Herrera, C.M. (2000) Flower-to-seedling consequences of different pollination regimes  
2 in an insect-pollinated shrub. *Ecology* **81**, 15-29.
- 3 Izhaki, I. & Safriel, U.N. (1990) The effect of some Mediterranean scrubland frugivores  
4 upon germination patterns. *Journal of Ecology* **78**, 56-65.
- 5 Jordano, P. (1992) Fruits and frugivory. *Seeds: The Ecology of Regeneration in Plant*  
6 *Communities* (eds M. Fenner), pp.105-156. CAB International, Wallingford,  
7 UK.
- 8 Krefting, L.W. & Roe, E. (1949) The role of some birds and mammals in seed  
9 germination. *Ecological Monographs* **19**, 284-286.
- 10 Lieberman, M. & Lieberman, D. (1986) An experimental study of seed ingestion and  
11 germination in a plant-animal assemblage in Ghana. *Journal of Tropical Ecology*  
12 **2**, 113-126.
- 13 Liu, H., Platt, S.G. & Borg, C.K. (2004) Seed dispersal by the Florida turtle (*Terrapene*  
14 *carolina bauri*) in pine rockland forests of the lower Florida keys, United States.  
15 *Oecologia* **138**, 539-546.
- 16 Lombardi, J.A. & Motta, J.C. jr. (1993) Seed dispersal of *Solanum lycocarpum* St. Hil.  
17 (Solanaceae) by the maned wolf, *Chrysocyon brachyurus* Illiger (Mammalia,  
18 Canidae). *Ciência e Cultura* **45**, 126-127.
- 19 Mandujano, S., Gallina, S. & Bullock, S.H. (1994) Frugivory and dispersal of *Spondias*  
20 *purpurea* (Anacardiaceae) in a tropical deciduous forest in Mexico. *Revista de*  
21 *Biología Tropical* **42**, 107-114.
- 22 Mas, R.E. & Traveset, A. (1999) Efectes de la ingestió per ocells sobre la germinació i  
23 la dispersió de dues espècies pròximes de *Solanum*. *Butlletí de la Societat*  
24 *d'Història Natural de les Balears* **42**, 67-77.
- 25 Mathsoft (1999) *S-Plus 2000. Guide to Statistics*, Vol. 2. Mathsoft Inc, Seattle.

- 1 Murphy S.R., Reid N., Yan Z.G. & Veneables W.N. (1993) Differential passage time of  
2 mistletoes fruits through the gut of honeyeaters and flowerpeckers. Effect on  
3 seedling establishment. *Oecologia* **93**, 171-176.
- 4 Murray K.G., Russell S., Picone, C.-M., Winnett-Murray K., Sherwood W. &  
5 Kuhlmann M.L. (1994) Fruit laxatives and seed passage rates in frugivores:  
6 consequences for plant reproductive success. *Ecology* **75**, 989-994.
- 7 Nogales, M., Delgado, J.D. & Medina, F.M. (1998) Shrikes, lizards and *Lycium*  
8 *intricatum* (Solanaceae) fruits: a case of indirect seed dispersal on an oceanic  
9 island (Alegranza, Canary Islands). *Journal of Ecology* **86**, 866-871.
- 10 Nogales, M., Valido, A., Medina, F.M. & Delgado, J.D. (1999) Frugivory and factors  
11 influencing visitation by birds at 'Balo' (*Plocama pendula* Ait., Rubiaceae)  
12 plants in the Canary Islands. *Ecoscience* **6**, 56-61.
- 13 Nogales, M., Medina, F.M., Quilis, V. & González-Rodríguez, M. (2001) Ecological  
14 and biogeographical implications of Yellow-Legged Gulls (*Larus cachinnans*  
15 Pallas) as seed dispersers of *Rubia fruticosa* Ait. (Rubiaceae) in the Canary  
16 Islands. *Journal of Biogeography* **28**, 1137-1145.
- 17 Olesen, J.M. & Valido, A. (2003) Lizards as pollinators and seed dispersers: an island  
18 phenomenon. *Trends in Ecology and Evolution* **18**, 177-181.
- 19 Paulsen, T.R. & Högstedt, G.H. (2002) Passage through bird guts increase germination  
20 and seedling growth in *Sorbus aucuparia*. *Functional Ecology* **16**, 608-616.
- 21 Rey, P. & Alcántara, J. (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea*  
22 *europaea*): connecting patterns of seed dispersal to seedling establishment.  
23 *Journal of Ecology* **88**, 622-633.
- 24 Sáez, E. & Traveset, A. (1995) Fruit and nectar feeding by *Podarcis lilfordi*  
25 (Lacertidae) on Cabrera Island (Balearic Archipelago). *Herpetological Review*  
26 **26**, 121-123.

- 1 SAS Institute Inc. SAS/STAT. Guide for Personal Computers, Version 8.02 Edition.  
2 Cary, NC: SAS Institute Inc., 2001.
- 3 Santamaría, L., Charalambidou, I., Figuerola, J. & Green, A.J. (2003) Effect of passage  
4 through duck gut on germination of fennel pondweed seeds. *Archiv für*  
5 *Hydrobiology* **156**, 11-22.
- 6 Schupp, E.W. (1993) Quantity, quality and the effectiveness of seed dispersal by  
7 animals. *Vegetatio* **107/108**, 15-29.
- 8 Traveset, A. (1998). Effect of seed passage through vertebrates on germination: a  
9 review. *Perspectives in Plant Ecology, Systematics and Evolution* **1**, 151-190.
- 10 Traveset, A., Gulías, J., Riera, N. & Mus, M. (2003) Transition probabilities from  
11 pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-*  
12 *salvatoris*) in two type of habitats. *Journal of Ecology* **91**, 427- 437.
- 13 Traveset, A., Riera, N. & Mas, R.E. (2001a) Passage through bird guts causes  
14 interspecific differences in seed germination characteristics. *Functional Ecology*  
15 **15**, 669-675.
- 16 Traveset, A., Riera, N. & Mas, R.E. (2001b) Ecology of the fruit-colour polymorphism  
17 in *Myrtus communis* and differential effect of birds and mammals on seed  
18 germination and seedling growth. *Journal of Ecology* **89**, 749-760.
- 19 Traveset, A. & Verdú, M. (2002) A meta-analysis of the effect of gut treatment on seed  
20 germination. *Seed Dispersal and Frugivory: Ecology, Evolution and*  
21 *Conservation* (eds D.J. Levey, W.R. Silva & M. Galetti), pp. 339-350. CAB  
22 International Publishing, Wallingford, UK.
- 23 Traveset, A. & Willson, M.F. (1997) Effects of birds and bears on seed germination in  
24 the temperate rainforest of Southeast Alaska. *Oikos* **80**, 89-95.

- 1 Verdú. M. & Traveset, A. (in press) Early emergence enhances plant fitness: a  
2 phylogenetically controlled meta-analysis. *Ecology*
- 3 Yagihashi, T., Hayashida, M. & Miyamoto, T. (1998) Effects of bird ingestion on seed  
4 germination of *Sorbus commixta*. *Oecologia* **114**, 209-212.
- 5 Witmer, M.C (1996) Do some bird-dispersed fruits contain natural laxatives? A  
6 comment. *Ecology* **77**, 1947-1948.

1 FIGURE CAPTIONS

2

3 **Fig. 1.** Final seed germination obtained for each plant species at the end of the study  
4 period, both in field and experimental garden conditions. Standard error bars are only  
5 shown in the first case as different stations were used as replicates (see details in text).  
6 Note that in some cases (such as the blackbird treatment in *Rubia* in), final seed  
7 germination was higher despite germination rate (speed) was lower.

8

9 **Fig. 2.** Index of germination rate of each species in the experimental garden and in field  
10 conditions, calculated from the parameter estimates and obtained from separate Cox  
11 regressions (Mean  $\pm$  SE). Data are expressed setting control treatments to zero. Data  
12 above and below zero indicate a higher or a lower, respectively, germination probability  
13 than the control treatment. Data from different species are not directly comparable, as  
14 estimates come from different regression curves. Differences between treatments were  
15 assessed using Chi-square test.; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; †  $P < 0.1$ .

16

17

1 **Table 1.** Main fruit characteristics for each study species (Mean  $\pm$  SD). Numbers in parentheses represent sample sizes. We consider fruit and  
 2 seed length as the maximum size of fruit and seed. Fruit and seed width were the perpendicular measure of fruit and seed length.

Species	Fruit length (mm)	Fruit width (mm)	Fruit weight (mg)	N seed per fruit	Seed length (mm)	Seed diameter (mm)	Seed weight (mg)	% Pulp water content
<i>Crataegus monogyna</i>	8.9 $\pm$ 0.6 (50)	8.1 $\pm$ 1.1 (50)	416.9 $\pm$ 142.8 (50)	1.1 $\pm$ 0.2 (50)	6.6 $\pm$ 0.8 (53)	4.9 $\pm$ 0.7 (53)	111.2 $\pm$ 46.5 (53)	66.56
<i>Ephedra fragilis</i>	6.6 $\pm$ 0.5 (50)	4.0 $\pm$ 0.6 (50)	55.5 $\pm$ 11.0 (50)	1.0 $\pm$ 0.0 (50)	6.0 $\pm$ 0.4 (50)	2.2 $\pm$ 0.2 (50)	16.7 $\pm$ 4.0 (50)	58.8
<i>Juniperus phoenicea</i>	10.0 $\pm$ 1.2 (50)	9.4 $\pm$ 0.9 (50)	653.7 $\pm$ 182.4 (50)	5.6 $\pm$ 1.4 (50)	5.6 $\pm$ 0.5 (150)	2.8 $\pm$ 0.3 (150)	27.3 $\pm$ 7.1 (150)	18.5
<i>Osyris alba</i>	9.8 $\pm$ 0.8 (50)	8.5 $\pm$ 0.8 (50)	633.0 $\pm$ 139.5 (50)	1.0 $\pm$ 0.0 (50)	6.7 $\pm$ 0.9 (50)	6.0 $\pm$ 0.9 (50)	149.9 $\pm$ 23.5 (50)	64.9
<i>Pistacia lentiscus</i>	4.8 $\pm$ 0.7 (50)	4.4 $\pm$ 0.4 (50)	56.4 $\pm$ 17.9 (50)	1.0 $\pm$ 0.0 (50)	4.4 $\pm$ 0.6 (50)	3.7 $\pm$ 0.5 (50)	16.3 $\pm$ 5.6 (50)	20.7
<i>Rhamnus alaternus</i>	6.3 $\pm$ 0.5 (50)	5.5 $\pm$ 0.6 (50)	129.2 $\pm$ 39.1 (50)	2.1 $\pm$ 0.1 (50)	4.6 $\pm$ 0.3 (68)	2.4 $\pm$ 0.3 (68)	9.5 $\pm$ 1.3 (68)	--
<i>Rhamnus ludovici-salvatoris</i>	5.4 $\pm$ 0.7 (210)	4.2 $\pm$ 0.6 (210)	84.9 $\pm$ 0.1 (210)	2.0 $\pm$ 0.4 (210)	3.8 $\pm$ 0.4 (210)	2.6 $\pm$ 0.2 (210)	14.2 $\pm$ 0.1 (210)	94.6
<i>Rubia peregrina</i>	4.9 $\pm$ 0.7 (50)	4.4 $\pm$ 0.7 (50)	105.8 $\pm$ 49.2 (50)	1.3 $\pm$ 0.5 (50)	3.8 $\pm$ 0.5 (62)	2.8 $\pm$ 0.3 (62)	31.1 $\pm$ 8.2 (62)	68.5
<i>Rubus ulmifolius</i>	11.5 $\pm$ 1.1 (50)	11.0 $\pm$ 1.6 (50)	993.2 $\pm$ 320.4 (50)	27.5 $\pm$ 10.3 (50)	2.7 $\pm$ 0.2 (180)	1.8 $\pm$ 0.2 (180)	4.2 $\pm$ 0.7 (180)	76.8
<i>Smilax aspera</i>	6.7 $\pm$ 0.7 (50)	5.9 $\pm$ 0.6 (50)	193.4 $\pm$ 43.7 (50)	2.4 $\pm$ 0.7 (50)	3.7 $\pm$ 0.3 (117)	3.4 $\pm$ 0.4 (117)	26.1 $\pm$ 8.9 (117)	70.7
<i>Solanum luteum</i>	9.5 $\pm$ 13.1 (50)	7.4 $\pm$ 0.5 (50)	258.8 $\pm$ 47.0 (50)	36.6 $\pm$ 8.2 (50)	1.9 $\pm$ 0.1 (250)	1.4 $\pm$ 0.1 (250)	34.8 $\pm$ 7.9 (48)*	83.2
<i>Solanum nigrum</i>	6.7 $\pm$ 0.6 (50)	6.7 $\pm$ 0.5 (50)	244.8 $\pm$ 41.8 (50)	38.8 $\pm$ 9.4 (50)	2.0 $\pm$ 0.1 (50)	1.5 $\pm$ 0.1 (50)	36.1 $\pm$ 6.2 (50)*	83.6

3 (\*) seed weight measurements were obtained by weighing groups of 20 seeds, due to the small seed size.

4

1 **Table 2.** Weight of seeds (mg; Mean  $\pm$  SE) in the two treatments (defecated by *Turdus*  
2 *merula* and control seeds, collected directly from the plant) for each tested species (data  
3 for *R. ludovici-salvatoris* not available) and percentage of seed mass increase of  
4 ingested treatment relative to control seeds. Numbers in parentheses are sample sizes.  
5 For the two species of *Solanum*, seed weight was obtained by weighing groups of 20  
6 seeds due to the small seed size.

7

Species	Control	Defecated	% Mass increase	F values
<i>Crataegus monogyna</i>	111.21 $\pm$ 6.38 (53)	92.83 $\pm$ 3.0 (183)	-16.5	$F_{1,234}=4.47$ *
<i>Ephedra fragilis</i>	15.02 $\pm$ 0.34 (140)	15.65 $\pm$ 0.24 (172)	4.2	$F_{1,310}=4.47$ *
<i>Juniperus phoenicea</i>	27.25 $\pm$ 0.68 (150)	22.71 $\pm$ 0.54 (150)	-16.7	$F_{1,298}=22.31$ ***
<i>Osyris alba</i>	149.89 $\pm$ 3.32 (50)	144.22 $\pm$ 1.70 (169)	-3.8	$F_{1,217}=2.10$ n.s.
<i>Pistacia lentiscus</i>	14.65 $\pm$ 0.43 (145)	12.91 $\pm$ 0.33 (148)	-11.9	$F_{1,291}=10.24$ ***
<i>Rubia peregrina</i>	30.86 $\pm$ 0.99 (65)	23.88 $\pm$ 0.61 (150)	-22.6	$F_{1,213}=33.20$ ***
<i>Rubus ulmifolius</i>	4.18 $\pm$ 0.05 (251)	4.48 $\pm$ 0.07 (149)	7.2	$F_{1,398}=11.28$ ***
<i>Smilax aspera</i>	26.08 $\pm$ 0.82 (117)	30.28 $\pm$ 0.83 (150)	16.1	$F_{1,256}=15.97$ ***
<i>Solanum luteum</i>	34.33 $\pm$ 1.12 (50)	27.52 $\pm$ 0.45 (22)	-19.9	$F_{1,70}=14.70$ ***
<i>Solanum nigrum</i>	36.06 $\pm$ 0.88 (50)	24.75 $\pm$ 0.53 (22)	-31.4	$F_{1,70}=67.24$ ***

8 \*\*\*  $P < 0.001$ ; \*  $P < 0.05$ .