
4 A Review on the Role of Endozoochory in Seed Germination

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Introduction

A large fraction of living plants produce fruits that attract animals (64% and 27% of gymnosperm and angiosperm lineages, respectively; Herrera and Pellmyr, 2002). In consuming them, animals can spread the seeds to more or less distant sites from the parent plant, thus contributing to plant regeneration and colonization of new sites (Willson and Traveset, 2000, and references therein). For millions of years both the pulp and seeds of fruit have been subjected to selective pressures exerted by frugivores and granivores. Fruit consumers, specifically, may show preferences for fruit traits such as size, shape, chemical composition and others, and have specific morphologies and physiologies of the digestive tract that affect the survival probability of the ingested seeds in different ways (Herrera and Pellmyr, 2002, and references therein). Seed traits are under further selective pressures imposed by a number of biotic (antagonistic fungi, insects and microbes; Cipollini and Levey, 1997) and abiotic factors (e.g. light, temperature, rainfall; Holl, 1999; Leishman *et al.*, 2000) that influence the dormancy period, germination time and/or future seedling growth, which can ultimately determine germination and seedling success (Verdú and Traveset, 2005, and references therein). Therefore, the specific seed traits in an endozoochorous plant are a result of the combined selection imposed by frugivores, granivores and other biotic and abiotic factors.

In this chapter, we evaluate the role of endozoochory on seed germination. We review the information gathered on germination patterns in experiments aimed at examining the effect of a seed's passage through a frugivore's gut, and give further directions on methods for future studies. Fruit treatment in the disperser's digestive tract (which includes pulp separation from seeds and treatment of the seeds) can determine the capacity of seeds to germinate and, thus, is one of the components of

disperser effectiveness that may be crucial for the population dynamics of many fleshy-fruited plant species. The nature of the relationships between fruit chemistry and morphology and the type of disperser is crucial to understanding the co-evolution of plant–frugivore interactions (e.g. Murray *et al.*, 1994; Cipollini and Levey, 1997; Wahaj *et al.*, 1998; Regan *et al.*, 2001; Tewksbury and Nabhan, 2001; Wenny, 2001; Stanley and Lill, 2002b; Alcántara and Rey, 2003).

Biology of the system: from fruit swallowing to seed fate

Frugivores that swallow fruits (subsequently referred to as frugivores) act differentially on seeds in several ways during ingestion, gut-processing and defecation. Plants, in turn, can be expected to evolve physical and chemical adaptations in fruits and seeds to direct dispersal and to maximize the proportion of seeds that are successfully dispersed and established (Table 4.1). Below we briefly outline the issues surrounding four main components of the process and follow this with detailed reviews in each area.

Removal of pulp surrounding the seed(s)

Fruit pulp often contains germination inhibitors that can block biochemical pathways of germination (e.g. Evenari, 1949; Mayer and Poljakoff-Mayber, 1982; Cipollini and Levey, 1997, and references therein; Meyer and Witmer, 1998). Moreover, the pulp can decrease and even preclude germination by

Table 4.1. Aspects of fruit processing during endozoochory likely to influence the germination of seeds when they are subsequently deposited.

	Pulp removal	Gut passage	Deposition pattern
Frugivore attributes	All frugivores adequate except fruit peckers	Large frugivores, especially mammals, have lengthy passage times and are chemically and mechanically harsh. Birds tend to be more gentle and pass seeds faster. Diet may have important effects.	Regurgitators tend to drop seeds singly but often close to the maternal tree. Large defecators produce dung with large numbers of seeds and high rates of seed mixing. Small frugivores deposit small numbers of seeds with less seed-mixing.
Fruit chemistry	Many plants appear to have germination inhibitors in the pulp that must be removed before germination will occur.	Passage rates may be influenced by secondary metabolites that act either as laxatives or constipating agents	Seeds may contain allelochemicals that inhibit the germination of competitors within the same faecal clump.

altering the seeds' microenvironment (e.g. osmotic pressure and light regime; Mayer and Poljakoff-Mayber, 1982; Meyer and Witmer, 1998; Samuels and Levey, 2005). The removal of fruit pulp and consequent release of the seed from this kind of inhibition (the deinhibition effect – Robertson *et al.*, 2006) has not received as much attention as scarification (the mechanical and chemical processing of the seed coat or endocarp), but the fact that seeds are more or less freed of pulp in the frugivore's gut is part of the seed passage process and thus needs to be considered in future studies, especially when the fate of uneaten fruits is considered (Samuels and Levey, 2005; Robertson *et al.*, 2006).

Mechanical and/or chemical scarification of the seed coat or endocarp

The effect of scarification is usually thought to depend on retention time in the digestive tract and on the type of food ingested along with seeds (Barnea *et al.*, 1990; Traveset, 1998). This mechanism (rather than deinhibition) is the most often invoked when comparing seed germination patterns between ingested and uningested seeds of the same species (Samuels and Levey, 2005). Despite this, we have very little direct empirical evidence of such alteration of the seed coat or endocarp structure.

Effect of the faecal material in which seeds are embedded at the time of deposition

The residues of whatever food items a frugivore has been eating can influence the microenvironment following defecation and may have a fertilizing effect on seedling growth (e.g. Dinerstein and Wemmer, 1988; Malo and Suárez, 1995; Traveset *et al.*, 2001a; Cosyns *et al.*, 2005). In some circumstances, however, the presence of faeces might reduce germination success by facilitating fungal and/or bacterial growth (Meyer and Witmer, 1998). In other cases, faecal material can protect seeds from attack of species-specific parasites and predators (Fragoso *et al.*, 2003). The rather sparse studies on this subject have reported that the material the dispersed seeds are embedded in is another factor that needs to be considered when evaluating the influence of frugivores on the final fate of seeds of fleshy-fruited plants.

The number and combination of seeds in the faeces

Seed-mixing may also influence germination and/or seedling growth. The number of seeds defecated in a dropping can be very relevant to the future establishment success of a plant. For instance, a mechanism of autotoxicity might explain the inverse correlation between seed abundance and germination success found by Barnea *et al.* (1992) for *Solanum luteum* and

Morus nigra. The presence of a large quantity of seeds in a dropping can also influence both post-dispersal seed predation (e.g. Janzen, 1986; Schupp, 1990; Traveset, 1990; Bermejo *et al.*, 1998) and intra- and interspecific competition (e.g. Loiselle, 1990). In addition, when different seed species are defecated in the same dropping, it is possible that one or more may exhibit allelopathic effects that inhibit the germination of the other species (Mayer and Poljakoff-Mayber, 1982; Cipollini and Levey, 1997).

Effect of frugivores separating pulp from seeds (deinhibition)

In drupes and berries, seeds are surrounded by a layer of flesh or pulp that offers a reward to frugivores but may also have other functions such as the protection of the seeds and the prevention of germination while still on the plant (Evenari, 1949; Mayer and Poljakoff-Mayber, 1982). This inhibition can take several forms. The inhibition may be induced by high osmotic pressure caused by the high sugar content of ripe fruits (Samuels and Levey, 2005), by light-blocking pigmentation that prevents enough light reaching the seedlings to stimulate germination (a possible reason why many ripe fruits are darkly pigmented; Cipollini and Levey, 1997), or from secondary metabolites that directly inhibit seed germination (Evenari, 1949; Cipollini and Levey, 1997). Although in most cases the exact mechanism is not known, many laboratory studies have confirmed that seeds often fail to germinate in Petri dishes when in intact fruit, while they readily germinate when hand-cleaned. For example, 60% of the 46 woody species that are dispersed by birds in New Zealand showed poor germination (<20%) in fruits. However, for most of these species, germination of hand-cleaned seeds was >90% (Kelly *et al.*, 2004). A recent review (Robertson *et al.*, 2006) showed that more than half of the species tested for deinhibition showed strong effects of removing the pulp on seed germination, though in most cases tests were conducted in Petri dishes, which appear to produce artificially large effects compared with tests conducted in the field (Rodríguez-Pérez *et al.*, 2005; Robertson *et al.*, 2006; see discussion below). To determine the extent to which the release of seeds from the pulp is a critical step in allowing germination, it is important that these tests are also conducted in field conditions, where chemical leaching and biological processing are quite different from those in the laboratory.

In a few cases, there is direct evidence of chemical inhibition of germination from constituents in the fruit pulp. Yagihashi *et al.* (1998) found that while seeds of *Sorbus commixta* (Rosaceae) that were hand-cleaned or that had been ingested and defecated by dusky thrushes (*Turdus naumanni*; Turdidae) germinated equally well in the laboratory, seeds that were either within intact fruits or extracted and treated with a 1% solution of pulp juice, failed to germinate at all. This suggests that a strong chemical which inhibits germination occurs in the pulp. The active

component or components were not identified in this case. Richmond and Ghisalberti (1994) found a similarly strong inhibition of seed germination in *Eremophila maculata* (Myoporaceae) from extracts of the fruit wall of *E. maculata* and *E. racemosa*. The active ingredient in this case was tentatively identified as an aromatic glycoside. Wahaj *et al.* (1998) fed artificial agar fruits containing *Solanum americanum* (Solanaceae) seeds and low concentrations of two naturally occurring *S. americanum* glycoalkaloids to cedar waxwings (*Bombycilla cedrorum*; Bombycillidae). The collected seeds from these fruits showed reduced germination rates compared with seeds collected following defecation from agar fruits that contained no glycoalkaloids. This suggests that these glycoalkaloids can maintain biological activity as germination inhibitors even after gut passage, and that they may have additional roles as allelopathic substances acting on other potentially competing seeds following deposition in faecal clumps. We will return to this idea when we consider deposition patterns.

Effect of frugivores on seed scarification

The seed coat is, to a variable extent, mechanically and/or chemically scarified when passing through the digestive tract of a frugivore. The level of scarification depends upon (i) the species of frugivore that ingests the fruit and (ii) the intrinsic traits of the fruits and seeds. The frugivores' morphological and physiological traits, as well as retention times in the gut, can alter the extent to which the seed coat is modified (e.g. Murray *et al.*, 1994; Traveset, 1998; Traveset *et al.*, 2001b, Figuerola *et al.*, 2002; Santamaría *et al.*, 2002; Charalambidou *et al.*, 2003, 2005; Pollux *et al.*, 2005). Important morphological traits include the length of the digestive tract and the presence of a gizzard; physiological traits include digestive fluids in the gut (which can vary depending upon the type of food ingested along with the fruits), water content, pH and proportion of plant material. Likewise, retention time can depend upon a variety of factors. Furthermore, plant traits such as pulp composition, seed age, seed size, coat thickness and texture also influence the degree of scarification. For instance, the pulp may have laxative compounds which promote a faster seed deposition (Murray *et al.*, 1994; Wahaj *et al.*, 1998) or constipative substances that slow it down (Cipollini and Levey, 1997).

A long retention time can be disadvantageous because the digestive fluids to which seeds are exposed may damage the embryo and thus decrease seed viability (Janzen *et al.*, 1985; Murray *et al.*, 1994; Santamaría *et al.*, 2002; Charalambidou *et al.*, 2005; Pollux *et al.*, 2005; but see Meyer and Witmer, 1998; Wahaj *et al.*, 1998). Because large seeds generally have shorter retention times in the gut (Levey and Grajal, 1991; Stanley and Lill, 2002a) we might expect their germination to be less affected by digestive fluids than that of small seeds. However, an interspecific comparison in a meta-analysis found that species with large seeds showed more effects of ingestion on germination (compared with hand-cleaned seed) than species

with small seeds (Traveset and Verdú, 2002), although this difference was no longer significant when the meta-analysis was phylogenetically controlled (Verdú and Traveset, 2005). Therefore, the implications of seed size for germination after passage through an animal's gut remain unclear and are probably species-dependent. Moreover, seed size is not always correlated with retention time in the gut, as recently found in the duck-dispersed pondweed, *Potamogeton pectinatus* (Potamogetonaceae; L. Santamaría *et al.*, unpublished results). More data are certainly needed both on the association between seed size and gut passage time and on the effect of such time on germination performance, especially for frugivores other than birds. Given the different food retention times that occur among different frugivore taxa (for instance, between small birds and non-flying mammals or reptiles), we might expect strong differences in the effect of seed size on retention time in the gut among these frugivore groups.

In a recent study that compared the effect of different guilds of frugivores on the germination of *Rubia fruticosa* (Rubiaceae), Nogales *et al.* (2005) found that seeds ingested by squirrels (*Atlantoxerus getulus*; Sciuridae) or rabbits (*Oryctolagus cuniculus*; Leporidae) were less viable and germinated in lower proportions than seeds ingested by either birds or lizards. They attributed this difference to the much longer retention time of seeds in the digestive tracts of the mammals. Seeds following frugivore gut passage had a much thinner coat than uningested seeds, presumably due to differences in the rate of seed-coat erosion (Fig. 4.1). In the Mediterranean species *Myrtus communis* (Myrtaceae), germination responses also differed according to the species of frugivore (Traveset *et al.*, 2001b). Again, these differences were attributed to the longer retention times of seeds in mammals compared with birds. However, the fruits of other species of plant ingested by different frugivores with very different retention times showed no differences in seed germination response (Traveset and Willson, 1997; Nogales *et al.*, 2005; Rodríguez-Pérez *et al.*, 2005).

A recent meta-analysis revealed that frugivore taxonomy has an important effect on seed germination responses (Traveset and Verdú, 2002). This analysis showed that bats and birds tend to have highly positive effects on seed germination (i.e. increasing germination percentages, compared with uningested seeds) whereas non-flying mammals have positive but small effects. Reptiles showed a non-significant effect, although the number of experiments with these frugivores is still small (Traveset and Verdú, 2002). Fishes could not be included in the analyses due to the scarcity of data on this group despite their importance as seed dispersers in many riverine systems. Such different effects among frugivore taxa are again attributed, at least partly, to the different food retention times, which is generally longer in non-flying mammals and reptiles than in birds and bats. Birds that include large amounts of fruits in their diet have a proportionally shorter intestine and a smaller and less muscular gizzard than non-frugivorous birds, which may result in a more gentle treatment of the coats of seeds ingested by specialist frugivores (Pulliainen *et al.*, 1981; Jordano, 2000). Morphological modifications are also found in

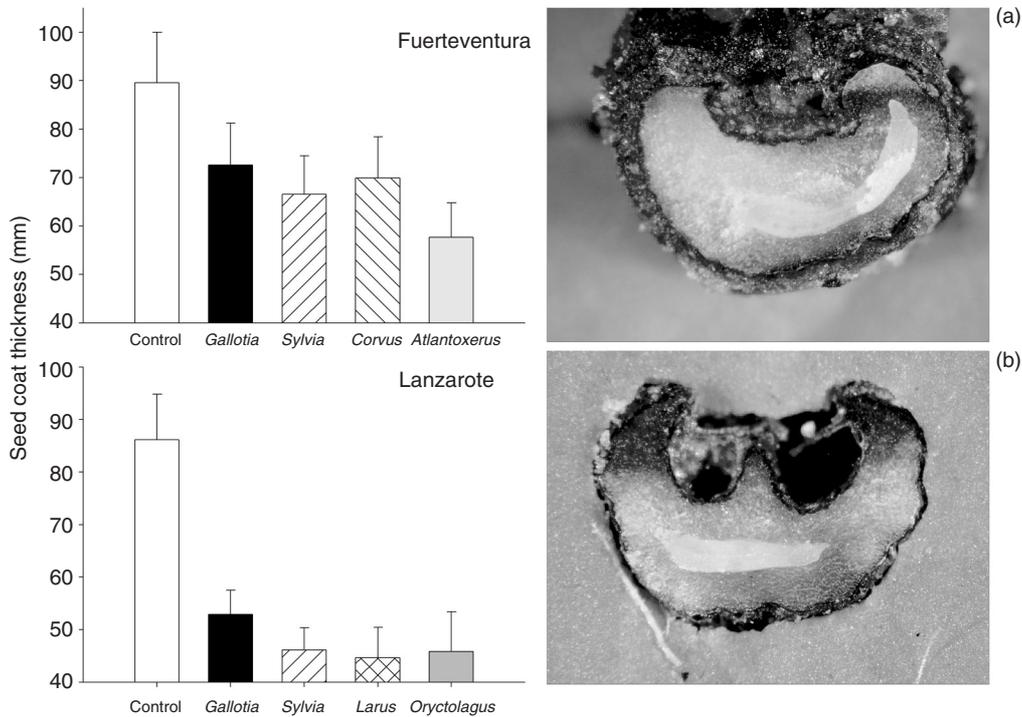


Fig. 4.1. Change in seed coat thickness found in *Rubia fruticosa* seeds after being ingested by different seed dispersers: lizards (*Gallotia atlantica*), warblers (*Sylvia* spp.), ravens (*Corvus corax*), squirrels (*Atlantoxerus getulus*), gulls (*Larus cachinnans*) or rabbits (*Oryctolagus cuniculus*) in (a) Fuerteventura and (b) Lanzarote (Canarian Archipelago). Cross-sections of a seed directly gathered from a fruit (a) and of a seed defecated by the squirrel (b) are shown. More details of the study can be found in Nogales *et al.* (2005).

frugivorous reptiles and bats; unlike birds, in these animals, frugivores generally have longer intestines than insectivorous species (Kunz and Ingalls, 1994; King, 1996).

Comparative tests of the scarification effect produced by different frugivores feeding on the same plant species often reveal large differences – some imparting a germination enhancement, others an inhibitory effect, and others a neutral effect (see review in Traveset, 1998). Likewise, the same species of frugivore may have different effects on germination depending upon the species of plant studied (Traveset, 1998; Traveset *et al.*, 2001c; Rodríguez-Pérez *et al.*, 2005). To further examine these patterns, we compiled two databases: one with a total of 29 studies in which percentage seed germination of a given species of plant was compared between at least two dispersers, and another ($n = 40$ studies) in which the effect of a disperser was compared, in the same study, with at least two different species of plant. For each database, a consistency index (dependent variable) was defined as the frequency of the most common effect (positive,

negative or neutral) divided by the total number of species tested (either disperser or plant, respectively). The independent factors were the species of disperser in the first database, and the species of plant in the second. The effect of different dispersers on the same plant was found to be more consistent than the effect of a single disperser over different plants. Consistent effects on germination (positive, negative or neutral) were observed in 68.8% of the cases comparing the effect of different dispersers on the same plant, and no significant differences in the consistency index when comparing frugivore groups; $\chi^2 = 3.23$, $df = 3$, $P = 0.357$). In contrast, consistency was much lower (33.8%) when a single disperser was tested with different plants ($\chi^2 = 22.8$, $df = 1$, $P < 0.0001$). These results imply that the particular seed characteristics of each plant (e.g. seed size, pulp composition, seed coat thickness, texture) promote more consistent responses than the specific morphological and/or physiological traits of frugivores.

Despite the fact that seed traits, rather than frugivore traits, dominate the effects of gut passage on germination response, frugivores also contribute to the heterogeneity of germination responses both within plant populations and within plant communities. This heterogeneity, with each species of frugivore having a particular effect on the seeds of each plant consumed, might be especially meaningful in unpredictable climates, such as those found in areas with unpredictable rainfall patterns. For instance, different studies in the eastern and western Mediterranean basin (Izhaki and Safriel, 1990; Barnea *et al.*, 1991; Traveset *et al.*, 2001b,c) have found that the seeds of a particular species of plant are differentially affected by passage through the guts of different bird species. Heterogeneity of seed responses occurs also at an intraspecific level. Meyer and Witmer (1998) reported individual differences within *Prunus virginiana* (Rosaceae) in germination responses, attributing them to differences in fruit nutrient composition as well as in the amount of fruit pulp accompanying the seed. A recent study performed with *Phillyrea angustifolia* (Oleaceae) and *Myrtus communis* with the goal of identifying mechanisms that can explain the different germination responses usually observed, showed that much inter- and intraspecific variation exists in different seed-coat traits. Such traits are: permeability, coat thickness, texture (measured by computing an index from a digital image obtained in the scanning electron microscope) and seed hardness (obtained by means of a testing machine (compression mode) that measures the load needed to break open the seed (A. Traveset, J. Rodríguez-Pérez and B. Pías, unpublished results); variation in all these traits has been found to be associated with variation in germination responses. This implies that, when carrying out studies to test the effects of endozoochory on seed germination, it is important to include in the tests seeds from a representative sample of individual plants. Less variation in germination has been found among individuals within a species of frugivore (A. Traveset, J. Rodríguez-Pérez and B. Pías, unpublished results), although it remains important to test germination using as many individual animals as possible.

Effect of manure/guano on germination and seedling growth: comparison among frugivores

The faecal material accompanying vertebrate-dispersed seeds at deposition sites can play an important role in enhancing seed germination and seedling survival (e.g. Dinerstein and Wemmer, 1988; Traveset *et al.*, 2001a). Although the fertilizing effect of such material has been acknowledged among ecologists concerned with seed dispersal by vertebrates (Willson and Traveset, 2000), little data exists that tests the size of this effect. Most frugivores have a very varied diet, which results in a highly variable manure composition; this is especially evident in 'carnivorous' mammals that seasonally consume great quantities of fruits (see, e.g. Herrera, 1989; Willson, 1993). The diet of bears, for instance, consists of vertebrates and invertebrates, as well as plant material such as fruits, stems, leaves or roots (Traveset *et al.*, 2001a). Bear manure consisting of animal material showed a consistently greater effect on seedling growth of *Vaccinium* spp. (Ericaceae) and *Rubus spectabilis* (Rosaceae) than bear manure consisting of vegetation fibre or fleshy pulp (Fig. 4.2; Traveset *et al.*, 2001a). While protein in the diet promotes a supply of nitrogen in the faeces, bones provide a source of calcium

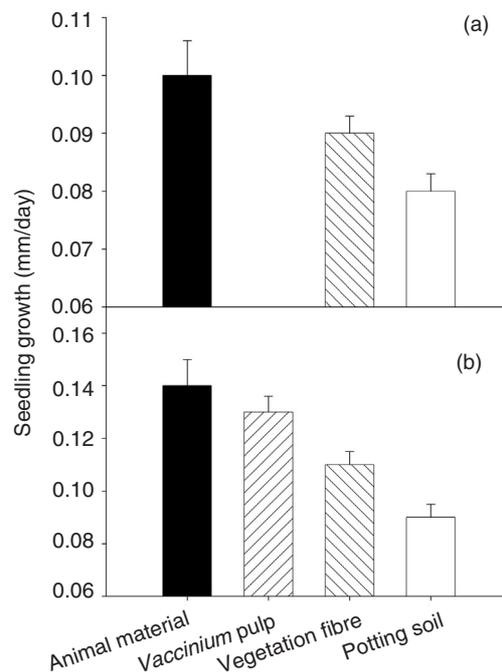


Fig. 4.2. Mean (\pm SE) seedling length of (a) *Vaccinium ovalifolium/alaskaense* and (b) *Rubus spectabilis* seedlings in different manure treatments. Potting soil was used as the control. Original Figures, drawn from data extracted from Traveset *et al.* (2001a).

phosphate which may limit growth in poor soils such as those in the temperate rainforest of Alaska (Willson *et al.*, 1998). Other components of the manure may supply some other nutrients or improve the moisture regime for seedlings, thus explaining the greater seedling growth in dung compared with the control (potting soil). Such results are consistent with those reported in other studies. Dinerstein and Wemmer (1988) found a significant increase in seedling growth of *Trewia nudiflora* (Euphorbiaceae) when seeds were embedded in rhinoceros dung compared with those growing on potting soil. Similarly, seedling growth of several *Acacia* (Mimosaceae) species in dung varied significantly between the species of consuming herbivore, probably owing to differences in the nutrient content and in the water-holding capacity of the dung (Miller, 1995).

As well as promoting seedling growth through a fertilizer effect, frugivore manure may also contain toxic compounds that can have negative effects on seed and/or seedling survival (Welch, 1985; Marambe *et al.*, 1993; Malo and Suárez, 1995; Cosyns *et al.*, 2005). Animal wastes are known to contain phenolic compounds and fatty acids that act as germination inhibitors for some plants. These compounds may alter the activity of enzymes that regulate the rate of germination (Marambe *et al.*, 1993, and references therein). Moreover, fruit pulp can also provide a substrate for the growth of fungi or bacteria, which can be very detrimental for germination or for seedling survival (Meyer and Witmer, 1998); thus, despite consuming large quantities of fruits, cedar waxwings were found to be ineffective dispersers of plants such as *Lindera benzoin* (Lauraceae) and *Prunus virginiana* (Rosaceae) because of faecal accumulation around seeds. In contrast robins, which usually regurgitate the seeds clean, did not have these negative effects on the germination of these species (Meyer and Witmer, 1998).

The difference in the influence of manure from frugivores belonging to different vertebrate taxonomic groups on seedling establishment success is likely to be great. The number and density of seeds in faeces, which is known to affect seed and seedling survival, is much higher in frugivorous mammals than in most birds (excepting cassowaries, emus and colonial nesting birds; A.J. Dennis, Queensland, 2005, personal communication) or reptiles (Barnea *et al.*, 1992; Willson, 1993; Andresen and Levey, 2004, and references therein). The large number of seeds in the dung of animals like rhinoceros, elephants or bears can attract seed predators, mainly rodents (Janzen, 1986; Traveset, 1990; Willson, 1993; Bermejo *et al.*, 1998; Andresen and Levey, 2004), and after germination, seedlings are likely to encounter intense competition (Lewis, 1987; Loiselle, 1990). On the other hand, the rich and copious droppings of a large mammal provide more nutrients to a seedling, over a longer period of time, than the small scats of most birds or lizards. In addition, birds excrete relatively high amounts of white urates when eating invertebrates, but watery material often coloured with fruit pigments when eating fruits. While it seems clear that the variation in faecal composition between dispersal agents might affect seed and seedling performance, little is currently known. Thus it would be useful to investigate this in future studies.

The effect of seed aggregations and seed mixing on germination (allelopathic and density-dependent effects)

Depending on a frugivore's size and behaviour, seeds that have been regurgitated or defecated will be deposited over a wide range of densities and with a varying degree of species-mixing (Stiles and White, 1986; Jordano, 2000; Fig. 4.3). Regurgitators tend to process fruits quickly and drop them one at a time beneath the maternal tree, with little species mixing; and the dropped seeds may accumulate to high densities (Stiles and White, 1986; Stiles, 2000). In contrast, fruit-ingesting frugivores deposit seeds in faecal clumps after gut processing. The degree of clustering of these seeds depends on the size of the faecal clump and the density of seeds in each clump, as well as on whether there is an aggregation of clumps beneath roosting or nesting trees, latrines or other regularly used locations (Debussche *et al.*, 1982; Debussche and Isenmann, 1994; Dean and Milton, 2000; Stiles, 2000; Takahashi and Kamitani, 2003). The degree of seed-mixing and the chance of deposition with heterospecific neighbours also vary with the size of the frugivore and the gut-retention time (Stiles and White, 1986; Jordano, 2000). Large animals tend to deposit clumps with more seeds and more seed-mixing than those of small frugivores.

Frugivorous birds typically deposit two or more species in droppings. Loiselle (1990) recorded an average of 132 seeds of 2.3 plant species per dropping in five species of small to medium-sized frugivorous passerines in Costa Rica. Stanley and Lill (2002a) found that in a temperate woodland in Australia, the white-eye (*Zosterops lateralis*; Zosteropidae) deposited on average 20–25 seeds of 1.1–1.3 species per dropping, and the superb fairy-wren (*Malurus cyaneus*; Maluridae) about 4–10 seeds of just one species. In contrast, one Cassowary dropping (*Casuaris casuaris*; Casuariidae) in North

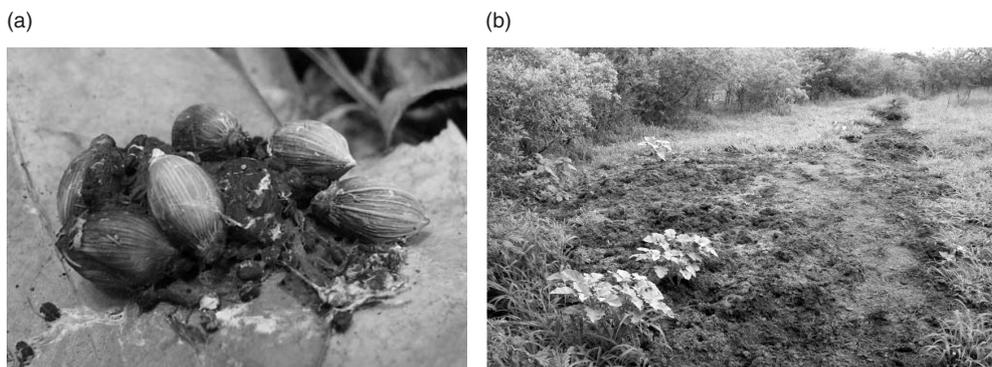


Fig. 4.3. Faecal clumps vary greatly in size from (a) bird droppings (from a New Zealand pigeon) with few seeds and a small amount of guano with one or two plant species, to (b) large mixed-species communal dung piles of large vertebrates like these white rhino latrines in South Africa with seedlings of the rhino-dispersed *Datura* sp. The environmental opportunities and challenges are obviously very different in their two extremes and are likely to have influenced the evolution of fruit and seed traits.

Queensland was found to contain 13 'more or less intact fruits' of *Beilschmiedia* sp. (Lauraceae), each with a diameter of about 6 cm and weighing around 52 g (Stocker and Irvine, 1983). The Asian rhino (*Rhinoceros unicornis*; Rhinocerotidae) disperses seeds in huge defecations that can weigh up to 23 kg and presumably potentially contain thousands of seeds (Dinerstein and Wemmer, 1988). Amazonian woolly monkey droppings were found to contain an average of 70 seeds of 2.53 species (Stevenson, Chapter 15, this volume). Pine and stone marten (*Martes martes* and *M. foina*, respectively; Mustelidae) scats collected from a woodland in Germany contained on average about 800 seeds per scat of a variety of fleshy-fruited species (Schaumann and Heinken, 2002). Thus, depending on the frugivore, deposited seeds may find themselves at low densities in the faeces or as part of a large assemblage of conspecific or heterospecific seeds competing for space, light and nutrients.

These very different environments pose different ecological opportunities and challenges, and if there is a consistent pattern to the deposition conditions, there may be selection pressures for the evolution of appropriate germination strategies (Linhart, 1976; Loiselle, 1990; Murray, 1998). In most cases, density-dependent factors will tend to impact negatively on the success of seedlings as they compete for resources and space (Lewis, 1987; Loiselle, 1990), so selection will tend to be for tactics that give an advantage to seedlings in this competition. Potentially these tactics may include early germination to beat the rush, or induced dormancy to wait out the crowd (Loiselle, 1990; Murray, 1998). Alternatively, seeds may produce allelopathic chemicals – or use those contained in fruit pulp – that are active against conspecific or heterospecific neighbouring seeds.

There is an extensive literature on the involvement of allelochemicals found in the seeds and fruits of dry-fruited species, particularly in agriculture. Plants that have been shown to produce water-soluble germination inhibitors that are potent against other pasture plants include the thistles *Carduus nutans* and *Onopordum acanthium* (Asteraceae; Wardle *et al.*, 1991; Qaderi *et al.*, 2003), Bishop's weed *Ammi majus* (Apiaceae; Friedman *et al.*, 1982), *Vigna mungo* (Fabaceae; Suman *et al.*, 2002) and *Lotus tenuis* (Fabaceae; Lattera and Bazzalo, 1999).

Apart from the evidence of pulp inhibitors mentioned earlier (which have all been tested on conspecifics), we are aware of only one study of a fleshy-fruited species that has demonstrated active allelochemicals that are effective on heterospecific seeds. Hruska *et al.* (1982) used a cucumber-seed bioassay to identify germination inhibitors from the lily *Liriope muscari* (Convallariaceae), although they did not test the effect of these inhibitors on seeds of species that are likely to be consumed by the same frugivores that disperse *L. muscari*, and the authors themselves interpret the data as evidence of auto-inhibition to prevent premature germination in-fruit rather than as evidence of allelopathy. Further tests of fruit constituents as sources of allelochemicals effective against potential competitors that are likely to co-occur within droppings would be welcome.

In some situations, there may be advantages to aggregated deposition patterns. D. Kelly *et al.* (unpublished results) deposited seeds of a New

Zealand mistletoe, *Peraxilla tetrapetala* (Loranthaceae), onto host branches as single seeds and in clumps of five. Germination and establishment success per seed was significantly higher in the clumps than in single seeds. This appeared to be due to an increased tendency to adhere to the host branch in clumps. However, over the subsequent 57 months, there was a tendency for slightly lower rates of survival in clumps, negating the initial advantage.

The consequences of the aggregation of seeds into clumps as the result of the feeding and deposition patterns of frugivores remains one of the least understood and studied aspects of seed dispersal. Characterizing the effectiveness of different frugivores by their seed dispersal kernels or by their ability to deposit seeds in safe sites ignores the potentially important effects that may occur at the scale of the individual dropping. Differential allelopathic and competitive interspecific effects between seeds within these clumps made by different frugivores have the potential to impose an additional layer of complexity that may distort the view established from simple movement patterns and gut passage times. However, until we have further studies that test the magnitude of these effects, it is difficult to assess their likely importance for the fate of seed.

How do we experimentally investigate the factors that influence seed fate?

Several key questions have emerged from the previous section concerning the interactions between frugivores and fruits and the way that these processes influence the success of seed germination. These questions are both ecological and evolutionary, have implications at the community level as well as for the demography of individual species and, we believe, should be of equal interest to theoretical and applied ecologists as well as to evolutionary biologists. Of central importance are the factors that influence the eventual fates of seeds, which can essentially be boiled down to these: (i) successful germination and establishment to the seedling stage, (ii) death by pre- or post-dispersal predation or disease, and (iii) the induction of dormancy and incorporation into the soil seed bank awaiting future opportunities. The likelihood of each of these is determined, at least in part, by the inherent biological properties of the plants' strategies shaped over millennia by natural selection and also by the changing biotic and abiotic environment and dispersal services provided by frugivores.

1. What happens to fleshy fruits if they are not ingested – are they doomed to fall from the plant and be prevented from germination by autotoxic inhibitors, or are they capable of germinating and maintaining the local population?
2. Does it matter what type of frugivores feed on the fruits? Do frugivores that regurgitate seeds produce different changes in seed traits relevant to germination than frugivores that defecate seeds?

3. How important is the composition of the droppings in which seeds are deposited? Is there interference between seeds in mixed-species droppings, and to what extent do the other constituents of the dropping facilitate or retard seed germination? We have come some way in determining the consequences of the different deposition patterns that are produced by different frugivores. For example, seeds in dense clumps are frequently worse off than those deposited in small groups, depending on the species. We now need to answer questions such as: (i) are early germination and allelopathy effective strategies for competing with neighbouring seeds; (ii) how important is the fertilization effect and does it alter the relative effectiveness of different dispersers?

Much progress has been made already and we have at least partial answers to some of these questions. However, we believe that further progress and clarification of inconsistent or uncertain results will come from careful attention to the design of experiments, as well as from attention to some factors that hitherto have largely been ignored. An important point that has emerged from the work done to date is the context in which experiments are performed. As we show below, conducting experiments in the field rather than in the laboratory is crucial for answering these questions if we wish to obtain answers that are relevant to the systems we study. In the following sections we outline six recommendations for the design of future experimental studies that seek to answer these questions.

1. Conduct these studies in field conditions

The micro-ecological conditions prevailing at the site where a seed is deposited can represent another source of variation in its future germination behaviour. Most reported information comes from studies carried out under controlled circumstances, usually in the laboratory (see review of the scarification effect in Traveset and Verdú, 2002), yet the results might not reflect what really happens in nature. Of the 83 studies reviewed by Traveset and Verdú (2002), 56% tested seed germination in laboratory conditions, another 22% performed germination experiments in the field, and 13% used glasshouses. Only a small fraction (7%) used more than one condition. The meta-analysis performed by Traveset and Verdú (2002) revealed that laboratory and field conditions were more likely than glasshouse experiments to detect differences between treatments (ingested vs non-ingested seeds), although the lower sample size for glasshouse cases might well have caused such differences. A more recent analysis of the scarification and deinhibition effects, which considered only bird-dispersal systems and included some additional studies that were not in the Traveset and Verdú (2002) dataset, suggested that, in general, the deinhibition effect is larger than the scarification effect, and that both effects are strongly influenced by the testing environment (Robertson *et al.*, 2006; Fig. 4.4). In this analysis, laboratory

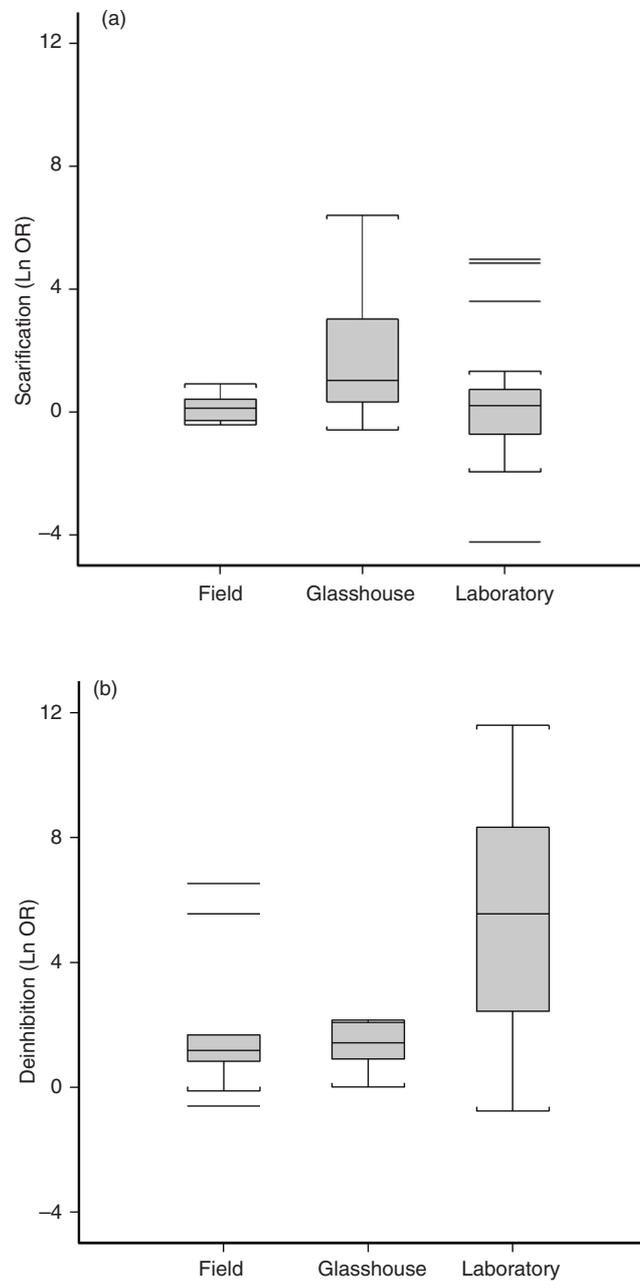


Fig. 4.4. Box plots of the relative size of (a) scarification and (b) deinhibition effects of fruit processing and the effects of the testing environment in fleshy fruited species. In (a) we calculated the size of the scarification effect by calculating the natural log (Ln) of the odds ratios (OR) comparing the final germination percentage of seeds from fruits passed through a gut versus those from hand-cleaned fruits in 56 species, following Traveset and Verdú (2002). In (b) we compared the effect of hand-cleaned versus intact fruits as a measure of the deinhibition effect in 51 species

studies tended to indicate stronger effects than either glasshouse or field trials, particularly for deinhibition. This might be due to the greater physico-chemical and biological processing of fruits and seeds that occur in the field after they have been deposited. This post-dispersal processing may replicate or replace the processes that happen in the animal gut to some extent and hence reduce the dependence on gut transport for successful germination (Morpeth *et al.*, 1997; Baskin and Baskin, 1998; Morpeth and Hall, 2000; Robertson *et al.*, 2006).

Studies that have tested the scarification effect in more than one experimental condition have often revealed rather inconsistent results (Bustamante *et al.*, 1992; De Figueiredo and Perin, 1995; De Figueiredo and Longatti, 1997; Yagihashi *et al.*, 1998; Traveset *et al.*, 2001c; Figueroa and Castro, 2002; Rodríguez-Pérez *et al.*, 2005; Robertson *et al.*, 2006). Although the constant conditions in the laboratory might seem ideal for detecting differences in germination between ingested and non-ingested seeds, this varies depending upon the plant tested (Table 4.2). Some studies have found a greater scarification effect on germination in the laboratory compared with field experiments (Table 4.2a; e.g. Bustamante *et al.*, 1992, 1993; De Figueiredo and Perin, 1995; Yagihashi *et al.*, 1998), while others have detected an effect of germination only in outdoor conditions and not in a growth chamber or glasshouse (e.g. Traveset *et al.*, 2001c). A few studies have found similar results between laboratory and field conditions (Rust and Roth, 1981; Braun and Brooks, 1987; Izhaki and Safriel, 1990; Barnea *et al.*, 1991). In a recent study, a total of eight species of plant were tested both in an experimental garden and in the field, and only half showed consistent results (Rodríguez-Pérez *et al.*, 2005). Overall, laboratory studies tend to show more benefit of scarification on germination compared with glasshouse or field tests, which on average show that scarification makes little difference to final germination percentage. There are very few cases where the deinhibition effect has been measured in more than one environment (Table 4.2b), but again these few results suggest that the results depends very much on the testing environment used.

The particular characteristics of the habitat where the germination tests are performed in the field may also affect the results. For instance, the salinity of the soil where seeds were sown was found to influence germination responses in two species of halophyte (Espinar *et al.*, 2004). Therefore, if our goal is to evaluate whether a change in germination outcomes is adaptive or not, it is crucial that seed responses to dispersers' gut treatment are examined in the field, in conditions that are similar to those encountered by the subject plant. The laboratory is useful for examining the underlying mechanisms once a field experiment has determined that the outcomes are important. Espinar *et al.*'s (2004) findings also highlight the need to consider the heterogeneity of the habitat in the experimental design, as this may be an additional source of variation in seed germination responses. Habitat effects have already been found in several studies on post-dispersal seed predation, germination success and

Table 4.2. Cases where the germination benefit of gut passage has been tested in more than one environment on replicate batches of seeds. The scarification effect compares hand-cleaned to defecated seeds, while the deinhibition effect is the comparison of hand-cleaned seeds to seeds in intact fruits. In both cases, the effect size is measured as the natural log of the odds ratio. Negative figures indicate a suppression of seed germination by either scarification or deinhibition, positive values indicate a germination promotion from these effects.

Plant	Family	Fruigrone	Scarification Ln OR			Authors
			Glasshouse	Field	Laboratory	
<i>Crataegus monogyna</i>	Rosaceae	<i>Turdus merula</i>	-0.04	-0.07	3.58	Rodríguez-Pérez <i>et al.</i> , 2005
<i>Cryptocarya alba</i>	Lauraceae	<i>Dusicyon culpaesus</i>		0.87	0.79	Bustamante <i>et al.</i> , 1992, 1993
<i>Ficus luschnathiana</i>	Moraceae	<i>Plathyrhinus lineatus</i>		0.32	0.83	De Figueiredo and Perin, 1995
<i>Ficus luschnathiana</i>	Moraceae	<i>Turdus leucomelas</i>		-0.02	0.18	De Figueiredo and Perin, 1995
<i>Gaultheria mucronata</i>	Ericaceae	Unidentified birds	0.30		0.69	Figueria and Castro, 2002
<i>Juniperus phoenicea</i>	Cupressaceae	<i>Turdus merula</i>		0.10		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Myrtus communis</i>	Myrtaceae	<i>Martes martes</i>	-0.24	-0.89		Traveset <i>et al.</i> , 2001a
<i>Myrtus communis</i>	Myrtaceae	Unidentified birds	-0.10	0.90	0.33	Traveset <i>et al.</i> , 2001a
<i>Osyris alba</i>	Santalaceae	<i>Turdus merula</i>	0.06	0.08		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Podophyllum peltatum</i>	Berberidaceae	<i>Terrapene carolina</i>	1.06	1.99	1.92	Rust and Roth, 1981; Braun and Brooks, 1987
<i>Rhamnus palaestinus</i>	Rhamnaceae	<i>Turdus merula</i>	-0.04	-0.46		Izhaki and Safriel, 1990; Barnea <i>et al.</i> , 1991
<i>Rubia peregrina</i>	Rubiaceae	<i>Podarcis pityusensis</i>	0.36	0.14		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Rubia peregrina</i>	Rubiaceae	<i>Turdus merula</i>	-0.48	0.07		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Rubia tenuifolia</i>	Rubiaceae	<i>Turdus merula</i>	-0.41	0.69		Izhaki and Safriel, 1990; Barnea <i>et al.</i> , 1991
<i>Rubus ulmifolius</i>	Rosaceae	<i>Podarcis pityusensis</i>	-0.49	0.12		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Smilax aspera</i>	Smilacaceae	<i>Turdus merula</i>	-0.15	0.20	-0.15	Rodríguez-Pérez <i>et al.</i> , 2005
<i>Solanum luteum</i>	Solanaceae	<i>Podarcis pityusensis</i>	0.04	0.20		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Solanum luteum</i>	Solanaceae	<i>Turdus merula</i>	0.59	-0.15		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Solanum nigrum</i>	Solanaceae	<i>Podarcis pityusensis</i>	0.59	0.12		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Solanum nigrum</i>	Solanaceae	<i>Turdus merula</i>	0.59	0.27		Rodríguez-Pérez <i>et al.</i> , 2005
<i>Sorbus commixta</i>	Rosaceae	<i>Turdus naumanni</i>		0.11	0.62	Yagihashi <i>et al.</i> , 1998
Average difference from field conditions			-0.10		0.49	

Plant species	Family	Deinhibition Ln OR			Authors
		Glasshouse	Field	Laboratory	
<i>Corynocarpus laevigatus</i>	Corynocarpaceae	3.58	1.06	6.66	Robertson <i>et al.</i> , 2005, Burrows 1996a
<i>Beilschmiedia tawa</i>	Lauraceae	1.00	1.00	6.98	Robertson <i>et al.</i> , 2005, Burrows 1999
<i>Ripogonum scandens</i>	Smilacaceae	20.04	4.06	8.50	Robertson <i>et al.</i> , 2005, Burrows 1996b
Average difference from field conditions		0.53		5.34	

seedling establishment (e.g. Herrera *et al.*, 1994; Schupp, 1995; Rey and Alcántara, 2000; Traveset *et al.*, 2003; Schupp, Chapter 20, this volume).

Placing seeds into the field requires a technique that allows the fate of seed to be easily followed. Robertson *et al.* (2006) used small sections of pipe partly pushed into the litter layer and laid out in a regular array to allow easy checking of germination and easy recovery of seeds by sieving the tube contents at the end of experiment to check for viability. Other options for relocating larger seeds include tiny metal tags or monofilament wire tethers attached to the hard parts of seeds (see, e.g. McGuinness, 1997; Steele *et al.*, 2001; Li and Zhang, 2003). Forget and Wenny (2005) provide a review of techniques used to follow seed fate, some of which may be applicable to germination studies.

2. Recognize and appreciate the distinction between the speed of germination and the final germination percentage and avoid talking about the rate of germination

Germination performance may be measured both by the proportion of seeds that have germinated at the end of the study and by the speed with which seeds germinate. Confusingly, both have often been termed germination 'rates' and this should be avoided in future studies (Robertson *et al.*, 2006). While the former is positively related to fitness, the second is not necessarily, as for instance, early germinated seeds might be more likely to die of desiccation, pathogens, predators, etc (Traveset, 1998, and references therein). Seeds that do not germinate immediately, or that enter dormancy, may nevertheless contribute significantly to plant fitness by dispersing in time rather than in space and may remain in waiting for suitable conditions (Kelly *et al.*, 2004; Robertson *et al.*, 2006). It is therefore worthwhile describing both the distribution of timing of germination as well as recording the final percentage of seed germination and checking for dormant seeds in the remainder.

3. Measure the deinhibition effect as well as the scarification effect

Several recent papers have noted the confusion in the literature about the processes that occur during gut passage, and have emphasized the need to measure both outcomes of gut passage: the deinhibition effect as well as the scarification effect (Kelly *et al.*, 2004; Samuels and Levey, 2005; Robertson *et al.*, 2006). If we are to answer the first two questions (**1** and **2**) from the previous section, we need to compare the performance of seeds from three types of treatment – (i) manually extracted from the pulp, (ii) intact fruits, and (iii) seeds that have been ingested and regurgitated or defecated by frugivores. Comparing (i) and (iii) is a test of the scarification effect, while comparison of (i) and (ii) is a test of the deinhibition effect. Comparing (ii) and (iii) allows an assessment of the combined effect of both processes. In

Samuels and Levey's (2005) review of 99 studies, 77% of these considered only the scarification effect, none measured simply the deinhibition effect, and only 18% included all three treatments and could therefore estimate the importance of both effects. The remaining 4% compared seeds from intact fruits and those defecated by frugivores and so were not able to separate deinhibition and scarification effects. The survey of the relative effect size by Robertson *et al.* (2006) suggests that the deinhibition effect is often larger than the scarification effect, although since most studies were conducted in Petri dishes which do not appear to accurately predict the effects in the field, further comparisons are needed from field conditions.

4. Allow sufficient time for seeds to germinate, and recover ungerminated seeds at the end of the study to check for viability/dormancy

As indicated above, seeds have three fates following dispersal – death, germination or secondary dormancy. Distinguishing between dormant and dead seeds is important, since the former may allow seeds to enter the soil seed bank (Kelly *et al.*, 2004) and so they should not be assumed to be dead (Baskin and Baskin, 1998; Robertson *et al.*, 2006). Some seeds may take several years to germinate. For example, seeds of the New Zealand gymnosperm *Prumnopitys ferruginea* (Podocarpaceae) were still germinating in field conditions more than 4 years after bird ingestion (Clout and Tilley, 1992). Seeds that are recovered may be tested for viability either with tetrazolium chloride (Cottrell, 1947) or placed in ideal laboratory conditions for germination.

5. Consider the influence of other components in the diet of frugivores

The diet of a particular species of disperser is known to alter food retention time. For instance, European starlings (*Sturnus migratorius*; Sturnidae) showed decreased gut passage times when their diet was changed from insects to fruits (Karasov and Levey, 1990). Likewise, a seed-based, high-fibre diet has been found to increase seed digestibility in mallards (*Anas platyrhynchos*; Anatidae) and germination of seeds of *Potamogeton pectinatus* decreased as seed retention time in the ducks' guts increased (Charalambidou *et al.*, 2005). For waterfowl, grit quantity (strongly related to diet) in the gizzard can also have large effects on seed germinability (e.g. Santamaría *et al.*, 2002). Thus, for future investigations on the effects of seed ingestion by dispersers on germination, it is important to maintain animals on the same diet when performing experiments in captivity. When defecated seeds are gathered in the field, the manure composition should be considered as a covariate, particularly in cases where it is variable. Transit time of seeds in the digestive tract needs to be considered in future experiments in captivity, especially if it is highly variable and ranges from several days to weeks.

6. Examine the mechanisms by which ingested seeds have a different germination performance from uningested seeds

Seed traits such as weight, coat permeability, coat thickness, texture and hardness may be related to either germination rate or germinability, or to both. For example, Nogales *et al.* (2005) found that *Rubia fruticosa* seeds ingested by squirrels showed the largest reduction in seed coat thickness and at the same time had the lowest viability. Due to variation in traits shown among individuals within species of dispersers and plants (A. Traveset, J. Rodríguez-Pérez and B. Pías, unpublished results), the use of known seed sources (maternal plants) and known individual dispersers will probably shed light on the mechanisms that can explain the differences in seed responses within a species.

7. Experiment with the deposition matrix

A critical aspect of the influence of the frugivores on seed germinability, which has until now been virtually ignored, relates to the nature of the deposition matrix that seeds find themselves in (Stiles and White, 1986; Jordano, 2000). Future studies should recognize that seeds are typically deposited in faecal clumps or droppings and are embedded in guano or other faecal matter and are often competing with a mixture of seeds from one or more species. Carefully designed studies that experiment with the components of this matrix will allow us to consider effects such as the fertilizer effect, seed mixing, the presence of germination inhibitors and/or allelochemicals, and the advantages of rapid or delayed germination in the clump. We know virtually nothing about the relative importance of these effects; however, for plant species that are dispersed by large frugivores that deposit large, highly mixed clumps, as well as by small animals with small droppings with few, unmixed seeds, these effects may be very important for comparisons of disperser effectiveness. All of them could be examined experimentally by following the fate of planted cleaned seeds in different faecal matrices, trying different combinations of neighbours and seed densities (Loiselle, 1990). Distinguishing between allelopathic and direct competition is not straightforward, but the use of stabilized seed extracts or purified secondary metabolites (Cipollini and Levey, 1997) or activated carbon to absorb leachates (Nilsson, 1994) may help shed light on the active mechanisms.

Concluding remarks

The study of how frugivores influence the germination patterns of endozoochorous plant species is of great relevance to our understanding of these animal-plant interactions from the ecological and evolutionary perspectives. From the plants' viewpoint, we need to know how the array

of frugivores that disperse their seeds determine: (i) the speed of germination and the percentage that germinate, which will depend upon fruit and seed processing in the digestive tract (among other factors); (ii) the seed deposition pattern, influenced by the number of seeds and species combination in the deposition (among other factors); and (iii) the plant establishment success within a community, which is partly a function of the type of material in which seeds are embedded at the time of deposition. Most data demonstrating the different mechanisms by which frugivores can affect seed fate are from studies performed with birds and mammals, which are the numerically dominant dispersers in many systems. In contrast, much less information has been gathered from other taxa, such as reptiles or fish. Likewise, we also need more data from field experiments, as most studies have performed germination tests under controlled conditions.

In this chapter, we have reviewed the most relevant information on this topic. From this review we have made a series of methodological recommendations and suggestions for new research directions. We hope these will be useful for those that continue to work on this line of research as well as for those young investigators who are willing to delve into this fascinating aspect of plant–animal interactions.

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