22 A Meta-analysis of the Effect of Gut Treatment on Seed Germination

Anna Traveset[1] and Miguel Verdú[2]

[1]*Institut Mediterrani d'Estudis Avançats (CSIC-UIB), C/ Miquel Marqués 21, 07190-Esporles, Mallorca, Balearic Islands, Spain;* [2]*Centro de Investigaciones sobre Desertificación (CSIC-UV), Camí de La Marjal s/n, Apdo. Oficial, 46470-Albal,Valencia, Spain*

Introduction

The dispersal of seeds by vertebrate frugivores is a process that usually implies the consumption of fruit pulp and the regurgitation or defecation of viable seeds (Ridley, 1930). An important advantage of seed ingestion by frugivores is a presumed increase in germination percentage (germinability) and rate (speed) (Krefting and Roe, 1949; van der Pijl, 1982). Recent analyses show, however, that such enhancement is far from universal and that several variables intrinsic to the plant or to the frugivore can influence the response of seeds to gut treatment (Traveset, 1998).

Three mechanisms determine how frugivores can directly affect seed germination: (i) possible mechanical and/or chemical scarification of the seed-coat, which may depend upon gut retention time and on the type of food ingested with seeds (e.g. Agami and Waisel, 1988; Barnea *et al.*, 1990, 1991; Izhaki and Safriel, 1990; Yagihashi *et al.*, 1998); (ii) separation of seeds from pulp because germination is reduced or precluded if seeds remain associated with pulp (e.g. Rick and Bowman, 1961; Temple, 1977; Izhaki and Safriel, 1990; Barnea *et al.*, 1991; Engel, 2000; Traveset *et al.*, 2001); and (iii) the effect that results from faecal material surrounding the seeds, which may influence germination and/or future seedling growth. For example, seedlings emerging from ingested seeds tend to be more vigorous than those emerging from uningested ones because remaining faecal material fertilizes the seedlings, especially in the case of large-mammalian faeces, which often take a long time to decompose (e.g. Dinerstein and Wemmer, 1988; Quinn *et al.*, 1994; Ocumpaugh *et al.*, 1996; Paulsen, 1998; Traveset *et al.*, 2001; T.R. Paulsen, unpublished).

The importance of the first mechanism – the modification of seed-coat traits (e.g. permeability of the coat to water and gases) after gut treatment, which changes the capacity of germination and/or the speed at which seeds germinate – is the focus of this study. A review of this effect has recently been published (Traveset, 1998). In this review, Traveset analysed the results of studies on nearly 200 plant species in 68 families, using a slight variation of the 'vote-counting' method (Light and Pillemer, 1971; Hedges and Olkin, 1980). This method is conservative and has the advantage of being simple, but it has the disadvantage of

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low statistical power (Cooper, 1998). Another shortcoming of the method is that all study cases are given the same weight, and thus the magnitude of the effect is the same for all studies. This equal weighting can generate biases when the number of seeds and replicates varies tremendously among studies. Thus, in this chapter we explored the effect of gut treatment on germination using a statistically powerful approach, meta-analysis, which combines results from independent studies and which is not sensitive to sample-size effects.

Meta-analysis requires the extraction of a common metric of effect size from each study included in the analysis. Choosing the best metric is of crucial importance for obtaining correct ecological inferences (Osenberg *et al.*, 2000). Estimates of effects from individual studies are combined into a pooled estimate of the overall strength of the effect, which is then used to assess significance (e.g. Xiong and Nilsson, 1999; Gurevitch *et al.*, 2000; Osenberg *et al.*, 2000).

Our analysis includes studies reviewed in Traveset (1998), plus studies that were missed in that review or were published more recently. We tested the following hypotheses:

1. Seed passage through a frugivore's gut increases both the germinability of seeds and the rate of germination.
2. Different taxonomic groups have different effects on seed germination. We expect such differences to result from differences in:
(a) seed retention times in digestive tracts, and/or
(b) chemical composition of the food ingested with the seeds.
3. Seeds from fleshy-fruited and dry-fruited species differ in their response to gut passage. This hypothesis assumes that pulp texture generally affects seed retention time in the gut (Levey, 1986) and, ultimately, germination patterns.
4. Large seeds will be affected differently from small seeds by gut passage, given that seed size affects retention time in the gut (e.g. Levey, 1986; Levey and Grajal, 1991; Gardener *et al.*, 1993), which presumably determines the degree of seed-coat scarification.
5. The effect on germination will vary depending upon plant life-form. Different life-forms have different frequencies of seed dormancy (Baskin and Baskin, 1998), which might reflect differences in traits such as coat thickness or sculpture.
6. Differences among habitats (cultivated areas, grasslands, shrublands and woodlands) may differ in the magnitude of the frugivores' effect on germination. As with the previous hypothesis, seed dormancy frequencies might vary depending upon the ecological conditions of the habitat where the species usually live (Baskin and Baskin, 1998, Ch. 12).
7. The effect of gut passage on germination differs between temperate and tropical plant species. This hypothesis is based on the prediction by Izhaki and Safriel (1990) that germination enhancement is adaptive in unpredictable or less constant environments.
8. Because the studies used to test the above hypotheses were conducted under a wide range of conditions (laboratory, greenhouse, field), we also tested whether the experimental conditions affected the probability of finding a significant effect.

The meta-analysis we performed, as with any type of statistical analysis, was limited to the data sets available. We have information on seed responses to gut passage (in particular, germinability data) from more than 100 frugivore species belonging to approximately 50 families, but these data are somewhat biased towards studies performed with either birds, mostly from the temperate zones, or non-flying mammals. Other frugivore groups are underrepresented (e.g. bats, reptiles and fishes). We do not include fish studies in our analyses because of the especially small number of studies available. Likewise, most data are from fleshy-fruited species; little is known about the responses of seeds in dry fruits to the passage through frugivores' digestive tracts.

Methods

We used results of 351 experiments, from 83 studies covering a total of 213 plant species, all of which considered seeds removed from pulp as 'controls' and used a minimum of 24 seeds. 'Treated' (ingested) seeds were typically seeds that had been defecated, except for several

cases in which data were given for regurgitated seeds only. We excluded studies in which it was not clear whether ingested and control seeds came from the same population and those that presented no sample sizes. Most studies provided only the final percentage germination and not the germination rate. When graphs of cumulative germination were presented, the rate was obtained directly from the curves. The entire database is available from the authors upon request.

We first calculated an effect size for each experiment. Because the end-point of seed germination experiments is binary (germinated versus non-germinated), we considered the log-transformed odds ratio (lnOR) to be the most appropriate metric of effect size (Egger *et al.*, 1997). Data were organized in a 2×2 contingency table; columns represented treatments and rows represented possible outcomes (Table 22.1). The odds of an event (i.e. seed germination) are the probability of the event occurring divided by the probability that the event does not occur. This odds ratio estimates the probability of germination after gut passage, relative to its probability in the control group. It was calculated following the Mantel–Haenszel procedure (Rosenberg *et al.*, 2000).

We next combined individual effect sizes, weighted by the reciprocal of their sampling variances, into a cumulative effect size representing the overall magnitude of the effect of gut treatment on seed germination, using the Mantel–Haenszel procedure (Rosenberg *et al.*, 2000). This estimate of effect size and its variance allows us to calculate confidence intervals (CIs) and the total heterogeneity, Q_T, of effect sizes (Rosenberg *et al.*, 2000). The effect of gut treatment on seed germination percentage is considered significant if zero is not included in the CI of the cumulative effect size. The heterogeneity among the n individual effect sizes was tested with the statistic Q_T against a χ^2_{n-1} distribution. A significant Q_T means that the variance among effect sizes is greater than expected from sampling error, and indicates that there may be underlying structure to the data and, therefore, that other explanatory variables should be taken into account.

If Q_T is statistically significant, the next step is to incorporate the data structure in the meta-analysis by including mediating variables (predictors). To test the hypotheses formulated in the Introduction, our predictors were: (i) the taxonomic group of the frugivore (birds, non-flying mammals, bats and reptiles); (ii) fruit type (dry and fleshy); (iii) seed size (small (< 5 mm), medium (5–10 mm) and large (> 10 mm)); (iv) plant life-form (herb, shrub and tree); (v) habitat (cultivated, grassland, shrubland and woodland); (vi) zone (temperate and tropical); and (vii) experimental condition (laboratory, field and greenhouse). A cumulative effect size with its variance and 95% CI was calculated for each group within a given predictor. Also, the heterogeneity within the group was tested with the statistic Q_W, against a χ^2_{n-1} distribution. A significant Q_W means that further heterogeneity remains unexplained within that group. To test differences in effect sizes among groups, the total heterogeneity Q_T was partitioned into between-group heterogeneity (Q_B) and within-group heterogeneity (Q_W). A significant Q_B means there are differences among groups in their cumulative effect sizes, also noted by non-overlapping 95% CIs (Rosenberg *et al.*, 2000). Fixed-effects analyses were used for all statistical tests.

A single study often contained several experiments. Therefore, experiments within a single study may be interdependent, leading to pseudo-replication. To control for this bias, we conducted an additional meta-analysis at the

Table 22.1. Contingency table representing the arrangement of data on seed germination for the calculation of odds ratios.

	Ingested	Control	Total
Germinated	A	B	A + B
Non-germinated	C	D	C + D
Total	$n_t = A + C$	n_c = B + D	N = A + B + C + D

A, B, C and D represent the number of seeds observed in each cell.

study level by pooling all experiments within a single study (see Xiong and Nilsson, 1999, for a similar procedure). Thus, each study contributed only one degree of freedom to the analysis, except in the few cases where experiments were reported for more than one frugivore group (i.e. bats and birds) and the experiments were pooled for each frugivore group. Predictors were assigned to each study only when more than 80% of the experiments had the same predictor (for example, when nine out of ten experiments from a given study were performed with small-seeded species).

Because some unpublished studies were included in the meta-analysis, a graphical method was performed to detect possible publication bias (e.g. if studies showing a significant effect of gut treatment on seed germination have greater probability of being published than those showing non-significant effects). We used a weighted histogram, which plots the effect size of the experiments against their weighted frequency (weights = 1/variance). If publication bias exists, the histogram will be depressed around the region of no-effect size.

Only three published studies (Barnea *et al.*, 1991; Iudica and Bonaccorso, 1997; Mas and Traveset, 1999) and unpublished studies by A. Traveset *et al.* had replicates to obtain means and standard deviations of the speed of germination, measured as time elapsed until reaching 50% germination (T_{50}). These studies contained a total of 30 experiments with 16 plant species. The effect-size metric for the study of the effect of gut treatment on germination rate was Hedges' *d*. This metric is an estimate of the standardized mean difference, unbiased by small sample sizes (Hedges and Olkin, 1985). We rejected an alternative metric, the response ratio, because effect sizes calculated with it were not normally distributed (Hedges *et al.*, 1999). Because of the small number of studies reporting data appropriate for a meta-analysis of germination rate, it was not possible to run an additional meta-analysis at the study level to control for pseudo-replication. For this reason, results of this meta-analysis must be interpreted with caution. We performed another meta-analysis on germination rate with a non-parametric estimate of the variance (i.e. variance calculated with sample sizes instead of standard deviations) (see Rosenberg *et al.*, 2000). In this analysis we could include 27 more experiments (and thus had a total of 57 observations). Effect sizes weighted by the non-parametric variance were bootstrapped 5000 times to estimate the 95% CI. All meta-analyses were run on METAWIN 2.0 (Rosenberg *et al.*, 2000).

Colinearity among variables was investigated by constructing a weighted least-squares multiple-regression model, following modifications proposed by Hedges (1994) for meta-analysis. Effect size, weighted by the inverse of its variance, was included as the dependent variable and predictors (transformed into dummy variables) were the independent variables. This model was run (SPSS 9.0) in a stepwise form to test the significance of each predictor, while controlling for effects of all other predictors. To determine significance, the difference between the sum of squares of the regression with (Q_R) and without (Q_r), the predictor ($Q_R - Q_r = Q_{\text{partial}}$) was tested against a chi-square distribution.

Results

Effect on final percentage germination

The overall effect of gut treatment on seed germination percentage was significantly positive (mean effect size lnOR = 0.29; 95% CI: (0.27–0.31)). The magnitude of this effect is considered 'moderate' in the social sciences (Cohen, 1988). A similar value was found when meta-analysis was carried out at the study level (lnOR = 0.25; (0.23–0.27)), which avoids pseudo-replication effects. The heterogeneity test of Q_T was highly significant ($P < 0.00001$), implying that other variables (predictors) account for some of the variation among studies.

When taxon is included as a predictor, the heterogeneity between groups, Q_B, is strongly significant ($P < 0.00001$), indicating that the effect of gut treatment on seed germination percentage differs among taxa. As shown in Fig. 22.1, all taxa except reptiles (whose 95% CI contains zero) have significant and positive effects on seed germination percentage. Taxa with overlapping 95% CIs do not differ

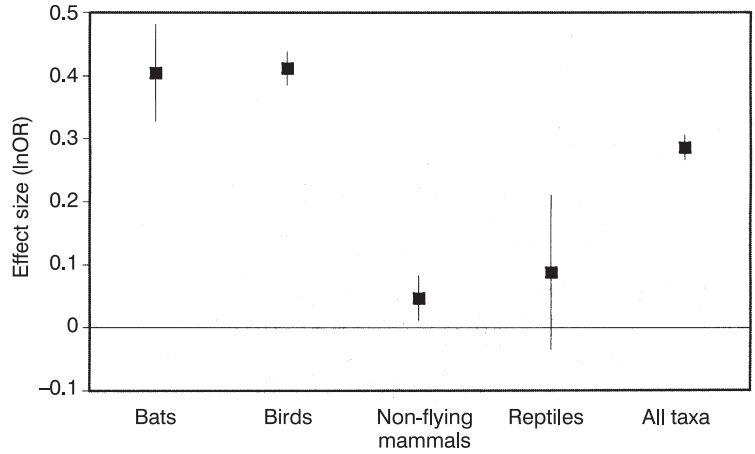

Fig. 22.1. Effect size of seed passage through the digestive system of bats (n = 19 experiments), birds (n = 180), non-flying mammals (n = 113), reptiles (n = 39) and all taxa (n = 351) on percentage germination. Effect size is calculated as the logarithm of the odds ratio (lnOR). Error bars indicate 95% confidence interval. An effect size is statistically significant if its error bar does not intersect the zero line. Two taxa are significantly different if their confidence intervals do not overlap.

statistically in effect size. Therefore, taxa may be grouped into two groups: (i) bats and birds, which have a similar effect size; and (ii) non-flying mammals and reptiles, which have little or no effect on seed germination percentage, respectively. The same groups were found when meta-analysis was carried out at the study level, although the effect of non-flying mammals shifted from being significant to being non-significant.

When considering fruit type, significant differences ($P < 0.00001$) were found between fleshy and dry fruits. While seeds from fleshy fruits ingested by frugivores germinated in higher percentages than control seeds, germination of seeds from dry fruits was negatively affected by gut treatment (Fig. 22.2a). The same results were found when meta-analysis was performed at the study level.

Seeds of all sizes were positively affected by gut treatment, although to different degrees ($P < 0.00001$). The effect on germination of large seeds was three to four times higher than the effect on small and medium seeds (Fig. 22.2b). Effect size for medium and small sizes did not differ. At the study level, the highest effect size was found for large seeds, but effect sizes for medium-sized seeds became non-significant and significantly lower than those for small seeds.

Frugivores had a more than four times stronger effect on seed germinability for trees than for shrubs or herbs ($P < 0.00001$) (Fig. 22.2c). When performing the meta-analysis at the study level, the strongest effect was still for trees and the effect for shrubs became non-significant.

Seeds from plants living in different habitats also responded differently to gut treatment ($P < 0.00001$) (Fig. 22.2d). The effect was positive for all habitats, but the magnitude of the effect ranged from strong to weak in the following order: cultivated > grassland > woodland > shrubland. The same order was found in the meta-analysis at the study level, although the effect for shrublands became significantly negative.

The effect of gut treatment on percentage germination was twice as large in the tropics as in the temperate zones ($P < 0.00001$) (Fig. 22.3a). This result was the same when the meta-analysis was performed at the study level. This finding, however, appears to be generated by the biased proportion of trees represented in tropical studies (80%). When we analysed the data comparing only seeds from trees, we found that temperate species were significantly more affected by gut treatment than were tropical species (lnOR = 0.75 vs. lnOR = 0.51, with positive and non-overlapping CIs) (Fig. 22.3b).

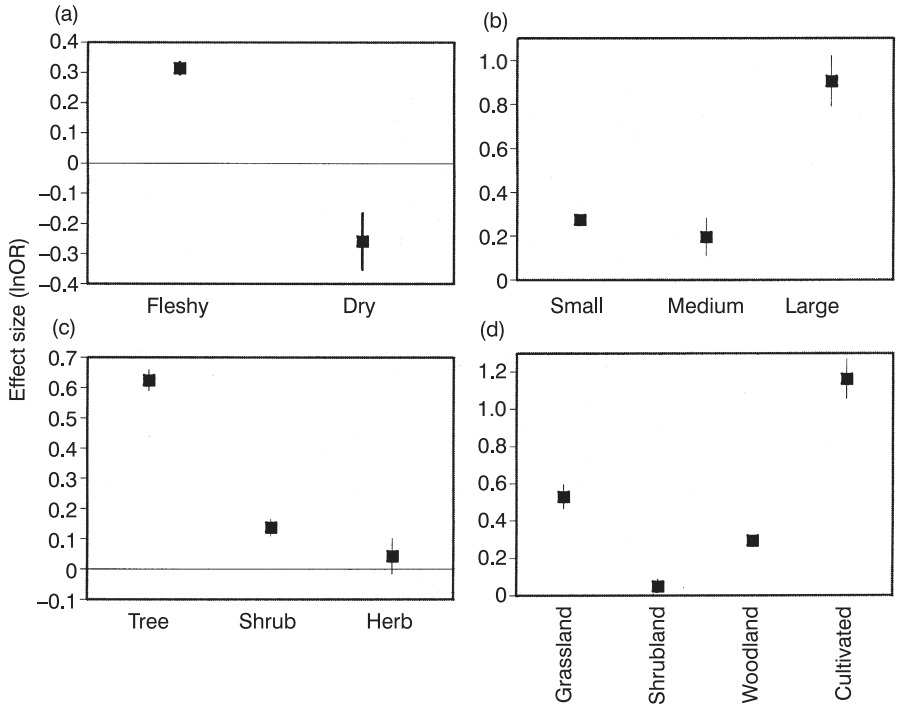

Fig. 22.2. Effect size of seed passage through frugivores' guts on percentage germination of seeds (a) from fleshy (n = 319 experiments) and dry (n = 32) fruits; (b) small (n = 253), medium (n = 43) and large (n = 37) sizes of seeds; (c) from trees (n = 116), shrubs (n = 154) and herbs (n = 66); and (d) from plants inhabiting grasslands (n = 25), shrublands (n = 79), woodlands (n = 214) and cultivated areas (n = 25). Data are mean effect sizes (logarithm of the odds ratio (lnOR)) and 95% confidence intervals.

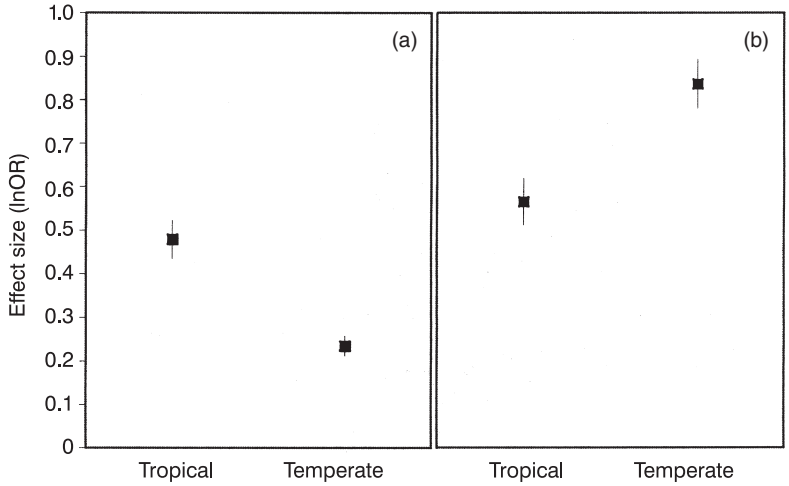

Fig. 22.3. Effect size of seed passage through frugivores' guts on percentage germination of seeds from (a) plants living in tropical (n = 82 experiments) and temperate (n = 269) zones, and (b) only trees tested in the two zones. Data are mean effect sizes (logarithm of the odds ratio (lnOR)) and 95% confidence intervals.

The experimental conditions under which seed germination was tested also influenced the results significantly ($P < 0.00001$) (Fig. 22.4). Experiments performed in the laboratory (usually in growth chambers) or in the field showed similar effect sizes, but greenhouse experiments seemed not to detect gut-treatment effects. The meta-analysis performed at the study level gave the same results, although field studies showed a slightly greater effect size than laboratory studies.

In all cases, the heterogeneity within a group Q_W (i.e. bats, small seeds, tropical zones, etc.) was statistically significant, suggesting that, even though our predictors explained a portion of the total variance, another fraction remains unexplained within groups. The regression model including all predictors was highly significant ($\chi^2 = 1339$; d.f. = 14; $P << 0.001$), although it only explained 13.5% of the variance. The error term was also highly significant ($\chi^2 = 5907$; d.f. = 275; $P < 0.001$) indicating that more predictors should be added to the model. All predictors remained highly significant after statistically controlling for the rest of the predictors, which indicates that the portion of the variance explained by each predictor is independent of that explained by the rest of the predictors (Table 22.2). Publication bias did not affect our analysis. The weighted histogram did not show smaller frequencies in the zone of no effect (Fig. 22.5).

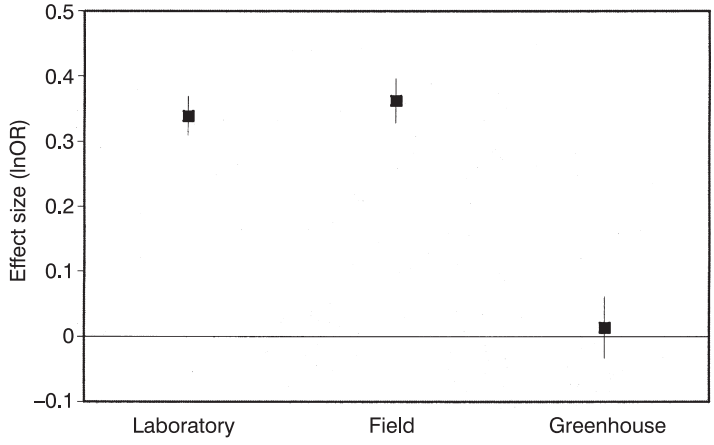

Fig. 22.4. Differences in the effect size of frugivores' guts on seed germination percentage among the three common germination conditions in which the experiments were run: greenhouse ($n = 57$), laboratory ($n = 179$) and field ($n = 115$). Data are mean effect sizes (logarithm of the odds ratio (lnOR)) and 95% confidence intervals.

Table 22.2. Results of the weighted regression model of effect size vs. predictors. The significance of each predictor after statistical control of the remaining predictors is tested. $Q_{partial}$ is calculated as the difference between the sum of squares of the regression with (Q_R) and without (Q_r) the predictor ($Q_R - Q_r = Q_{partial}$) and tested against a χ^2 distribution with the degrees of freedom shown in parentheses.

Predictor	Q_R (d.f.)	Q_r (d.f.)	$Q_{partial}$ (d.f.)	P
Frugivore taxa	1339.2 (14)	1230.5 (11)	100.8 (3)	10^{-23}
Fruit type	1339.2 (14)	1250.6 (13)	50.9 (1)	10^{-21}
Seed size	1339.2 (14)	1318.5 (12)	25.6 (2)	10^{-5}
Life-form	1339.2 (14)	1263.5 (12)	140.1 (2)	10^{-17}
Habitat	1339.2 (14)	953.6 (11)	243.1 (3)	10^{-83}
Zone	1339.2 (14)	1327.4 (13)	2.3 (1)	10^{-4}
Experimental condition	1339.2 (14)	1287.6 (12)	41.5 (2)	10^{-12}

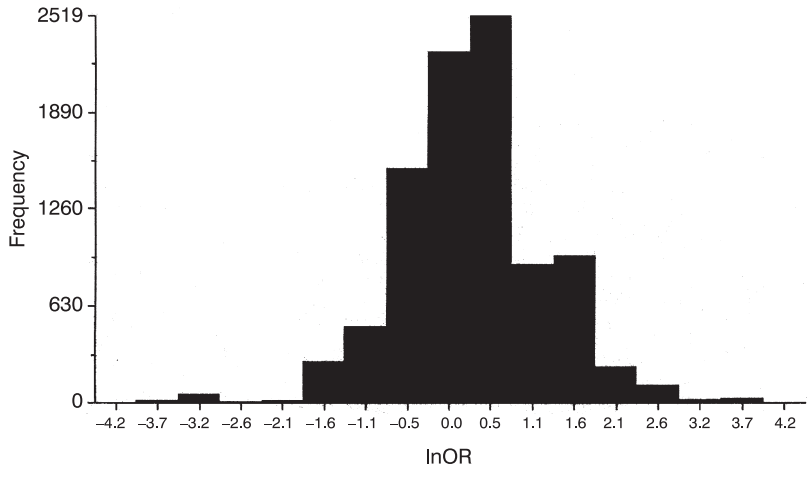

Fig. 22.5. Weighted histogram of effect size of the experiments against their weighted frequency (weights = 1/variance) to detect publication bias. lnOR, logarithm of the odds ratio.

Effect on rate (velocity) of germination

The speed at which seeds germinate was also significantly affected by gut treatment (Hedges' $d = -0.3843$; (−0.5716 to −0.1970)). The negative effect size means that the time needed for gut-treated seeds to germinate was lower than that for control seeds. Thus, frugivores significantly accelerated seed germination. The heterogeneity test, Q_T, was highly significant ($P < 0.00001$) and therefore other explanatory variables (predictors) should be investigated. This is not possible, however, because of the small number of replicated experiments reporting germination curves. Conclusions and limitations were the same when the sample size was increased from 30 to 57 experiments by performing a meta-analysis weighted by a non-parametric estimate of the variance (Hedges' $d = -6.73$; (−20.8 to −0.25) bias-corrected bootstrap 95% CI).

The difference between control and ingested seeds on germination rate (T_{50}) averaged only a few days. It was similar among the taxonomic groups (1.8 ± 14.7 days for birds, $n = 63$; 1.5 ± 14.8 days for non-flying mammals, $n = 55$; and −1.4 ± 1.0 days for bats, $n = 4$; means ± SD). Only one observation existed for reptiles; it showed a difference of 1 day. Differences in time to first germination (or dormancy length) between ingested and control seeds also averaged a few days (1.5 ± 16.8 days, $n = 66$); the range, however, was quite wide, from a delay (for ingested seeds) of 110 days to an acceleration of 37 days.

Discussion

What affects seed germination?

Our analysis confirms that seed passage through the digestive tract of vertebrate frugivores, regardless of taxa, influences germination. Ingested seeds germinate in greater numbers and take less time to germinate than uningested seeds. As expected, the magnitude of the effect differed significantly among frugivore groups. In contrast to Traveset's (1998) results, however, birds and bats had a significantly greater positive effect on germination than either non-flying mammals or reptiles. We attribute this result to the shorter gut-passage times, usually associated with body size (Karasov, 1990), in the former group compared with the latter. Moreover, birds and bats void the indigestible mass (seeds) as quickly as possible to reduce the high energetic costs of ballast during flight, a selective pressure that is nonexistent in non-volant vertebrates (Levey and Grajal, 1991). Seed retention time in the gut presumably determines the abrasive effect on the seed-coat, and we have some evidence showing that germination success decreases as retention time increases (Murray *et al.*, 1994). Besides having different seed retention times,

the two groups of frugivores may differ significantly in the chemical composition of ingested food (with variable acidity, water content, secondary compounds, etc.), which can also affect the degree of seed-coat scarification and, thus, germination (Murray *et al.*, 1994; Witmer, 1996; Cipollini and Levey, 1997; Wahaj *et al.*, 1998). The food's chemical composition can, in turn, affect gut passage time (Witmer, 1996; Wahaj *et al.*, 1998; Levey and Martínez del Río, 1999). A change in diet from insects to fruits, for instance, appears to decrease seed retention time in American robins, *Turdus migratorius* (Levey and Karasov, 1994).

We found a striking difference between the effect of gut treatment on seeds of fleshy and dry fruits; the effect was positive for the former but negative for the latter. This finding suggests that frugivores might act as a selective agent on the type (texture, chemical composition, etc.) of pericarp within which seeds are embedded. Endozoochory is in fact evolutionarily associated with the production of fleshy pulp. The negative effect found on seeds from dry fruits indicates that their ingestion by frugivores is not always beneficial to the plant. It would be interesting to know how seeds from fleshy and dry fruits differ in traits relevant to germination, such as coat structure and thickness, and in retention time in vertebrate guts.

The size of seeds proved to be important in determining the magnitude of the germination effect. Small seeds are often retained for longer periods in an animal's digestive tract than are large seeds (e.g. Garber, 1986; Levey and Grajal, 1991; Gardener *et al.*, 1993; Izhaki *et al.*, 1995) and thus they may be more likely to be excessively abraded. Seed-coat thickness, possibly associated with seed size, might also account for this finding.

Traveset (1998) concluded that seeds of trees were more frequently affected by gut passage than were seeds of other life-forms. Although this result was confirmed by our meta-analysis, the factors that produce it are unknown. It may be that some seed traits differ among life-forms, making them differently 'sensitive' to mechanical and/or chemical abrasion. Interestingly, a seed trait that varies in the same direction as the effect of life-form is dormancy. Trees in the temperate zone have a greater frequency of non-dormancy (51%) than other life-forms (42% for shrubs and 37% for herbs) (Baskin and Baskin, 1998).

When comparing habitat types, cultivated species appeared to be much more affected than species in the other habitats, regardless of life-form. The explanation for this result may be related to the fact that seed dormancy usually decreases in cultivated species (Baskin and Baskin, 1998). In fact, human selection may have caused a reduction in the thickness of seed-coat, which would probably mean a loss of dormancy (Baskin and Baskin, 1998).

If the effect of gut treatment on germination is in fact associated with seed dormancy, we might also expect that species in the tropics, with a lower dormancy frequency (Baskin and Baskin, 1998), would be more strongly affected than species in the temperate zone. However, the pattern we observed is the opposite, at least for trees. We have no explanation for this result.

Differences in germination percentage between ingested and uningested seeds may depend on the type of experimental conditions. The probability of detecting a difference appeared to be greater when seeds were planted in the natural habitat (or simply outdoors) and when simply placed in Petri dishes than when planted in a greenhouse. In the few studies that performed the same test under different conditions, either a greater effect in laboratory compared with field experiments (Bustamante *et al.*, 1992, 1993; Figueiredo and Perin, 1995; Yagihashi *et al.*, 1998) or a similar result between the two conditions was found (Figueiredo and Perin, 1995; Figueiredo and Longatti, 1997). Traveset *et al.* (2001) performed simultaneous tests in the three conditions and found similar results in the laboratory and greenhouse, detecting differences between ingested and control seeds only in the field. Other studies on plant responses to particular effects have also reported significantly different results depending on environmental conditions (e.g. Curtis and Wang, 1998).

Adaptive significance of germination enhancement

The enhancement of germination of seeds passed through a frugivore's gut can be

adaptive only if it translates into an increase in plant fitness. High germinability is presumably necessary to maximize reproductive success and seedling recruitment (Harper, 1977). An acceleration of germination, in contrast, may not always be beneficial, and can even be detrimental if other factors affecting seedling recruitment (pathogen attack, herbivory, water limitation, light conditions, etc.) favour dormancy (e.g. Janzen 1981; Jones and Sharitz, 1989). The potential advantages of fast germination probably vary among species, depending on the type of seed dormancy they have and on the ecological conditions prevailing in the habitat. Non-dormant species might be expected to benefit from fast germination more than dormant species, because such a response might reduce mortality, due to factors such as seed predation (e.g. Schupp, 1993), sibling competition (e.g. Hyatt and Evans, 1998) or shade intolerance (Jones *et al.*, 1997). For dormant species, the risk of mortality may be spread over time to maximize plant fitness, by being differently affected by each vertebrate frugivore that consumes their fruits (Izhaki and Safriel, 1990; Barnea *et al.*, 1991).

Seed passage through the gut of a vertebrate can probably break only seed-coat dormancy (so-called functional dormancy) and not physiological (internal or embryological) dormancy, as differences in germination rate between ingested and uningested seeds are usually only a few days and more rarely a few weeks. Such a relatively short period is unlikely to result in important differences in seedling survival, as recently confirmed with *Myrtus communis* by Traveset *et al.* (2001).

Avenues of Future Research

Given that seed germination responses can vary significantly due to a variety of factors, both extrinsic and intrinsic to the plant, future tests on the effect of gut treatment on germination need to be tightly controlled. In particular, control seeds should be collected from the same populations where fresh faeces are gathered and at the same time. When performing experiments with captive frugivores, we recommend that fruits be mixed and randomly sampled before being fed to the frugivores; this will ensure that seed traits of the two groups (control and treatment) differ minimally. Due to the differences we documented among different environmental conditions, we also recommend the tests be done in the species' natural habitat. If that is not possible, it would be prudent to perform tests in more than one condition. To avoid planting seeds that appear intact but are not viable, seed viability should be tested prior to germination trials.

The speed of germination, and not simply the final percentage germination, should be more closely examined, as differences may exist in the former that could pass undetected. We also recommend that germination be monitored for a period long enough to allow most seeds to germinate.

Our data set shows that we especially need much more information on the effect of gut treatment on: (i) seeds from dry fruits; and (ii) seeds from life-forms other than trees in the tropics. We also need more studies that examine the effect of bats and reptiles on seed germination.

It is also of crucial importance to determine the mechanism/s by which seed germination behaviour is modified after seeds are ingested by an animal. Future research should investigate the changes that seed-coat traits (thickness, porosity, etc.) undergo after seeds pass through a frugivore's gut. It would also be interesting to know how often seed dormancy is related to seed-coat thickness (Baskin and Baskin, 1998) and how this affects the probability of germination enhancement after gut treatment.

Finally, to elucidate the consequences of seed ingestion by frugivores for plant recruitment and, ultimately, the consequences of frugivory for plant reproduction, we need more studies that focus on the transition stage from seed to seedling. Moreover, the importance of seed ingestion by frugivores on germination performance and plant fitness can only be determined by evaluating the effect of all other factors, biotic and abiotic, that influence germination and establishment success.

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