PLANT ANIMAL INTERACTIONS

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Bridging meta-analysis and the comparative method: a test of seed size effect on germination after frugivores' gut passage

Abstract Most studies using meta-analysis try to establish relationships between traits across taxa from interspecific databases and, thus, the phylogenetic relatedness among these taxa should be taken into account to avoid pseudoreplication derived from common ancestry. This paper illustrates, with a representative example of the relationship between seed size and the effect of frugivore's gut on seed germination, that meta-analytic procedures can also be phylogenetically corrected by means of the comparative method. The conclusions obtained in the meta-analytical and phylogenetical approaches are very different. The meta-analysis revealed that the positive effects that gut passage had on seed germination increased with seed size in the case of gut passage through birds whereas decreased in the case of gut passage through non-flying mammals. However, once the phylogenetic relatedness among plant species was taken into account, the effects of gut passage on seed germination did not depend on seed size and were similar between birds and non-flying mammals. Some methodological considerations are given to improve the bridge between the meta-analysis and the comparative method.

Keywords Effect size · Evolution · Frugivory · Seed size

Introduction

In the last two decades, the comparative method has experienced such theoretical and statistical development that it has become a standard tool in studies on

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evolutionary ecology aiming to establish the relationship between traits across taxa (Martins 2000). Several methods have been developed to establish relationships between traits taking into account the phylogenetic relatedness across taxa (Harvey and Pagel 1991). The validity of these methods for drawing inferences about adaptive processes from reliable phylogenies has produced a growing interest in the analysis, and re-analysis, of interspecific databases (e.g. Dodd et al. 1999; Larson and Barret 2000; Pagel 2000 and references therein).

Another statistical tool aimed at the analysis and re-analysis of databases is meta-analysis, which is expanding into ecological and evolutionary studies (e.g. Järvinen 1991; VanderWerf 1992; Møller and Thornhill 1997; Bender et al. 1998; Van Zandt and Mopper 1998; Byers and Waller 1999; Xiong and Nilsson 1999; Arnqvist and Nilsson 2000; Gillooly 2000; Gurevitch et al. 2000; Poulin 2000; Norby et al. 2001; Rustad et al. 2001; Searles et al. 2001; Traveset and Verdú 2002). Criticisms of meta-analysis have mainly focused on the problems of (1) publication bias (Jennions and Møller 2001; Kotiaho and Tomkins 2002), and (2) non-independence among observations (taxa) due to phylogenetic relatedness (Gurevitch et al. 2001). So far, although a large number of studies using meta-analysis have tried to establish relationships between traits across taxa from interspecific databases, these meta-analyses have not been "phylogenetically corrected" by means of any of the available methods (Poulin 2000; Jarosik et al. 2002; Traveset and Verdú 2002; but see Møller and Thornhill 1998).

The aim of our paper is to illustrate, with a representative example, that meta-analytic procedures can, and must, also be phylogenetically corrected by means of the comparative method. Our example examines the relationship between seed size and the effect of frugivores' gut passage on the probability of seeds germinating. We selected seed size as a working variable because this trait is strongly correlated with taxonomic membership (Mazer 1990; Jordano 1995).

Materials and methods

Data on the effect of seed treatment in frugivores' guts on germinability were those employed in Traveset and Verdú (2002), who meta-analyzed 351 experiments comparing final germination of ingested versus uningested seeds (control group). Rather than categorizing seed size, this time we obtained (either from the literature or by asking for this information directly to authors) quantitative values for this variable. We used maximum seed length (log-transformed to achieve normality) as this was the most frequent measure reported in the studies. We obtained these data from a total of 103 fleshy-fruited species: 58 of these were subjected to gut passage through birds and 45 through non-flying mammals. We excluded data from reptiles and bats, as well as data from dry fruits, due to the relatively few studies available on them. The entire database is available from the authors upon request.

The metric chosen for the effect size was the log-transformed odds ratio (lnOR), which estimates the probability of seed germination after gut passage, relative to its probability in the control group (Egger et al. 1997). Significant positive values of lnOR indicate that seed germination is enhanced by gut passage whereas significant negative values indicate the opposite. The 95% confidence interval of the effect size shows whether the value is significantly greater or less than zero. When the same plant species was present in several experiments, a cumulative effect size following the Mantel-Haenszel procedure (Rosenberg et al. 2000) was calculated to obtain just one effect size per species and thus avoiding pseudoreplication in subsequent analyses. Likewise, if the same plant species had been tested for both birds and non-flying mammals, we only considered the data reported for the frugivore group in which the experimental sample size (number of seeds) was highest.

A fixed effects meta-analysis of covariance (meta-ANCOVA hereafter) was carried out to test for the effects that bird and non-flying mammal's guts have on the germination of different-sized seeds. Random effects models are currently available for meta-analysis but not for comparative methods. Thus, to make comparable the analyses, we decided to use fixed-effects meta-ANCOVA.

Meta-ANCOVA is a particular case of the General Linear Model applied to meta-analysis consisting of a weighted least squares regression where the weight is the inverse of the variance of the effect size, and the resulting standard error of the regression coefficient is corrected by a factor of the square root of the residual mean square for the regression. Finally, the significance of the model is provided by the sum of squares of the regression contrasted against a chi-square distribution with the same degrees of freedom as the number of dependent variables included in the model (Hedges and Olkin 1985). Thus, the effect size (lnOR) was considered the response variable and it was weighted by the inverse of its variance; the frugivore group (birds vs. non-flying mammals) was the explanatory variable, while seed size was the covariate. The interaction between frugivore type and seed size informed us, as in a traditional ANCOVA, about the homogeneity of slopes (i.e. if the gut effect of both birds and non-flying mammals was similarly correlated with seed size). Because meta-analysis is not well developed to statistically account for different slopes, we had to perform separate analyses for birds and mammals after assessing such heterogeneity.

In order to incorporate the phylogenetic relationships of the plant species in our study, we ran a phylogenetical ANCOVA (phylo-ANCOVA hereafter) following the same design used for the meta-ANCOVA described above: the effect size (lnOR) was the response variable while frugivore type was the explanatory variable and seed size the covariate. The phylo-ANCOVA was run using PDAP software (Garland et al. 1993), contrasting the conventional F-values of the analysis of covariance (PDSINGLE module) against the values obtained from the phylogenetically correct, empirically scaled null distribution generated after 10,000 simulations of the evolution of the (possibly correlated) characters along the phylogenetic tree of the plants (PDSIMUL and PDANOVA modules). As we previously did in the meta-ANCOVA, we tested for homogeneity of slopes to know if the gut effect of the two groups of frugivores was similarly correlated with seed size. We checked if the weight that the meta-ANCOVA gives to the effect size may alter the results of the phylo-ANCOVA by re-running the phylo-ANCOVA with the gut effect on germination (lnOR) weighted (multiplied) by the inverse of its variance [var (lnOR)].

The plant phylogeny used to run the phylo-ANCOVA was that published in Soltis et al. (2000) with grafted clades (Rosaceae and Caprifoliaceae) resolving phylogeny at the genus level. Because phylogeny was unknown for several groups (e.g. *Ficus*, Araliaceae, Moraceae), our phylogenetic tree contained several polytomies. These "soft" polytomies, reflecting a lack of information about the branching pattern instead of a true instantaneous speciation, can be handled by correcting the degrees of freedom of the model (Garland and Díaz-Uriarte 1999). A super-tree was constructed with information from several phylogenies, and therefore equal branch lengths were assumed (Ackerly and Reich 1999; Verdú 2002). Equal branch lengths are appropriate under the punctuational evolutionary model, but may inflate the type I error rates (Ackerly 2000). We checked the standardization of branch lengths for both seed size and gut effect on germination by correlating the absolute value of each contrast versus its standard deviation (Garland et al. 1992). Furthermore, we tested several branch length transformations (i.e. Pagel's arbitrary and Grafen's arbitrary branch lengths) and obtained the same results, which makes our results more robust with respect to branch lengths. The pdi file containing both the phylogeny and the tip values is available from the authors upon request.

Results

Meta-analytic approach

Frugivores significantly improved the germination of seeds [(lnOR=0.26; 95% confidence interval (0.22–0.30)], and this effect was significantly different (Q_B=42.1; df=1; P<0.00001; heterogeneity test between groups) between birds [lnOR=0.19; (0.15–0.23)] and non-flying mammals [lnOR=0.45; (0.38–0.53)]. Heterogeneity within both frugivore groups was highly significant (Q_w=1,318.1 and Q_w=1,038.5 for birds and non-flying mammals, respectively; P<0.00001 in both cases), indicating that other variables should be included in the model to explain the variance. By including seed size (log-transformed) as a covariate in the meta-GLM model (meta-ANCOVA), we found that the slopes between the response variable (gut effect on germination) and the covariate (seed size) were different between birds and non-flying mammals (sum of squares of the interaction between seed size×frugivore group=Q_R=38.6; df=2; P<0.000001).

In the case of birds, the meta-analysis showed that the germination enhancing effect of gut passage through birds increased with increasing seed size (y=−0.15+0.37x; P<0.00001), although the percentage of explained variance was low (R^2=9.6%) (Fig. 1). In contrast, the germination enhancing effect of gut passage through non-flying mammals decreased with increasing seed size (y=0.61−0.14x; P<0.001), and in this case, the explained variance was negligible (R^2=1.4%) (Fig. 1).

In short, the meta-analysis revealed that the positive effects that gut passage has on seed germination increase slightly with seed size in the case of birds whereas they decrease negligibly in the case of non-flying mammals.
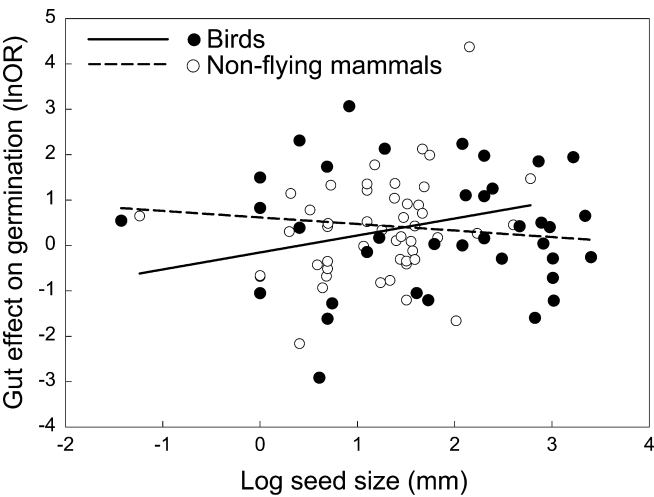
Fig. 1 Meta-analytic approach to the relationship between seed size and the frugivore's gut effect on seed germination. Birds and non-flying mammals are analyzed separately because of the heterogeneity of the slopes

Phylogenetic approach

Unlike the meta-ANCOVA, the phylo-ANCOVA showed that the slopes of seed size on gut effect on germination were not significantly different between birds and non-flying mammals ($F_{1,81}=2.07$; $P=0.22$). The same conclusion was reached when the gut effect was weighted by the inverse of its variance, as the meta-analysis does ($F_{1,81}=1.71$; $P=0.27$). Because the ANCOVA assumption of homogeneity of slopes was fulfilled, the effects of birds and non-flying mammals were studied together in the same model. The gut effect on germination was not different between birds and non-flying mammals and was not related to seed size (Table 1; Fig. 2). The results were the same when the weighted gut effect was used as the dependent variable (Table 1; Fig. 3)

Therefore, the phylogenetic analysis revealed that the effects of gut passage on seed germination do not depend on seed size and are similar between birds and non-flying mammals.

Table 1 Phylogenetic ANCOVA on the effects of frugivores group (birds vs non-flying mammals) and seed size on the unweighted and weighted gut effect on seed germination

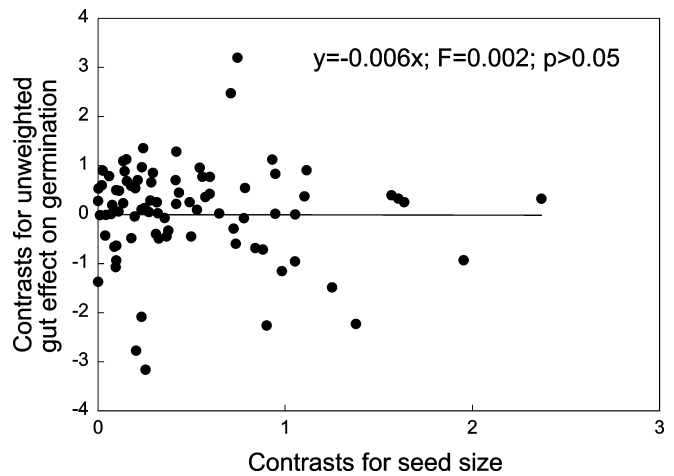| Unweighted				
Source of variation	SS	df	F	P
Frugivores	0.08	1	0.06	0.87
Seed size	1.05	1	0.72	0.77
Error	119.2	82		
Weighted				
Source of variation	SS	df	F	P
Frugivores	108	1	0.05	0.87
Seed size	375	1	0.17	0.88
Error	173,653	82		

Fig. 2 Phylogenetic approach to the relationship between seed size and the unweighted frugivore's gut effect on seed germination
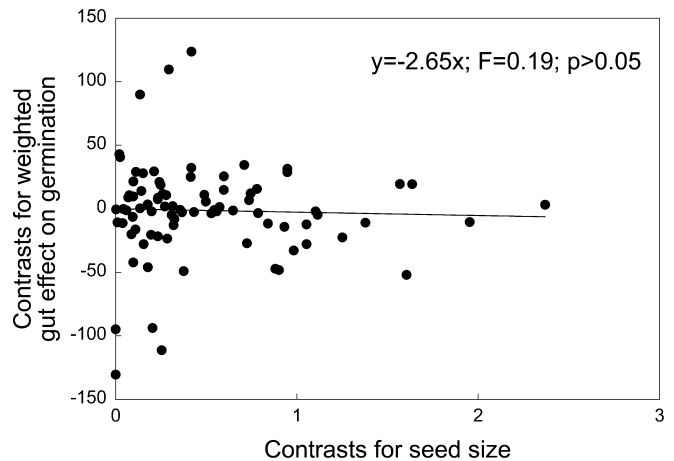

Fig. 3 Phylogenetic approach to the relationship between seed size and the weighted frugivore's gut effect on seed germination. The weight is the inverse of the variance. Birds and non-flying mammals are analyzed together because of the homogeneity of the slopes

Discussion

Bridging meta-analytic procedures with the comparative method, when taxa are considered as experimental units, has been shown to be very useful for extracting more reliable conclusions than when performing meta-analysis alone (Traveset and Verdú 2002). The present study illustrates this, considering the relationship between seed size and the frugivores' gut effect on seed germination. The most interesting finding in our analyses was that the conclusions obtained in the meta-analytical and phylogenetic approaches are very different.

For avian frugivores, a positive meta-regression between seed size and gut effect on germination was found. The percentage of intergeneric variance of seed size explained by the regression line was around 10%, close to that found in the multivariate meta-regression model which included seven independent variables to explain frugivores' gut effect on seed germination (Traveset and Verdú 2002). This indicated that germination of small

seeds is negatively affected when ingested by birds, but this effect becomes positive as seed size increases. An ecological explanation for this is that small seeds are retained for longer in a bird's digestive tract than large seeds (Levey and Grajal 1991), which may cause an excessive abrasion of the seed coat within the gut. Short transit times through the digestive tract have been interpreted as an adaptation that allows birds to consume fruits in greater quantities and, consequently, the fitness of the large-seeded plants might be increased. Alternatively, larger seeds could have thicker coats with the same effects (unfortunately, no data are available to test this possibility).

In the case of non-flying mammals, the meta-regression between seed size and gut effect on germination was negative, the effect of seed treatment in mammals' guts being positive on small seeds and negligible on large seeds. Such results actually support the hypothesis that mammals have exerted a selective pressure favouring small-seeded species, given that the mammalian syndrome consists of fruits with many small seeds (Herrera 1989). Alternatively, the mammalian syndrome may be related to the taxonomical composition of mammal-ingested fruits (Herrera 1987). The results of the present paper actually support the latter hypothesis because the significant relationship between seed size and mammals' gut effect on seed germination vanished after the phylogenetic control of plant species. Furthermore, the low percentage of variance explained by the meta-regression line (1.4%) suggests that this relationship is not biologically relevant.

The significant relationships that were found between seed size and frugivores' gut effect on germination disappeared after controlling for taxonomic relatedness among plant species, implying that the association between both characters is due to common ancestry and not to the result of correlated evolution. It is well known that a great amount of interspecific variation in seed size is accounted for by taxonomic relatedness (Herrera 1992). For example, Lord et al. (1995) have shown that taxonomic membership accounted for around 90% of seed mass variation in six data sets from different regions of the world. When considering only fleshy-fruited species, the amount of variance explained by the phylogeny of 910 world-wide species was only 32% (Jordano 1995). This means that even though phylogeny may have constrained the force of natural selection in shaping seed size, a considerable amount of variance is still available for natural selection to act upon. The results from our study suggest that the role of frugivores' guts on the evolution of seed size has been negligible. However, other factors linked to seed treatment in the guts of frugivores and seed size, such as emergence, growth and survival rates in seedlings born from seeds ingested by animals should be simultaneously investigated to know the importance of treatment in frugivores' guts on the evolution of seed size (Paulsen and Högstedt 2002).

The philosophy of modern meta-analysis is to combine the individual effect sizes by means of weighted statistical models, using as weight the inverse of the variance of the effect size (Rosenberg et al. 2000). In this way, when several effect sizes are averaged to calculate a cumulative effect size, greater weight is given to the effect sizes with smaller variances. In the comparative method, the weights used to calculate ancestral states are a function of the within-species variation, the phylogeny, and the model of character evolution (Martins and Hansen 1997). Therefore, we believe the comparative method would benefit considerably by including the weight used in meta-analysis for the estimation of ancestral states. This weight is a measure analogous to the measurement error derived from the individual variation within species, and the incorporation of that error into the phylogenetically independent contrasts does not affect point estimates of parameters (correlations between traits, values at ancestral nodes, *P*-values, etc) (Garland et al. 1993). In our analysis, the conclusions of the phylo-ANCOVA did not change after weighing effect sizes, but we argue that the statistical consequences of this procedure must be checked in each study.

A final consideration to apply to future studies combining meta-analysis and the comparative method is that ecological datasets usually involve two independent sets of relatives (in this case, plants and frugivores) and, thus, the analysis should account for the phylogeny of both. As far as we know, no comparative method is able to account simultaneously for two phylogenies from two different groups of species. In the present example, we have considered only the phylogeny of plants, which may confound effects if, for example, seed size covaries with frugivore taxon. In such a case, the effect of gut passage on germination may be covarying with the frugivore clade and not with seed size.

Further studies combining the comparative method and meta-analysis will certainly help to develop both methods in the field of evolutionary biology.

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