

ORIGINAL ARTICLE

Seed traits and taxonomic relationships determine the occurrence of mutualisms *versus* seed predation in a tropical forest rodent and seed dispersal system

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Abstract

Although many studies have been carried out on plant–animal mutualistic assemblages, the roles of functional traits and taxonomy in determining both whether interactions involve mutualisms or predation and the structure of such assemblages are unclear. We used semi-natural enclosures to quantitatively assess the interaction strengths between seeds of 8 sympatric tree species and 4 rodent species in a tropical forest in Xishuangbanna, Yunnan, Southwest China. We found 2 clusters of species in the seed–rodent network represented by 2 genera in the Fagaceae (*Castanopsis*, *Lithocarpus*). Compared to seeds of 3 *Castanopsis* species, seeds with heavy weight, hard coat or caloric content (including 3 *Lithocarpus* species) were eaten less and more frequently hoarded by rodents. In turn, hoarded seeds showed less predation and more mutualism with rodents. Our results suggest that seed traits significantly affected the hoarding behavior of rodents, and, consequently, the occurrence of mutualisms and predation as well as assemblage structure in the plant–animal seed dispersal system. Taxonomically-related species with similar seed traits as functional groups belong to the same substructures in the assemblage. Our results indicate that both seed traits and taxonomic relationships may simplify thinking about seed dispersal systems by helping to elucidate whether interactions are likely to be dominated by predation or mutualism.

Key words: hoarding behavior, mutualism, predation, seed dispersal, seed trait

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INTRODUCTION

Seed dispersal networks have been investigated in several different ecosystems (Jordano *et al.* 2007; Carlo & Yang 2011; Donatti *et al.* 2011). Careful analysis of seed dispersal mutualistic interactions between plants and animals reveals that these mutualisms seem to be the network of numerous, diffuse and asymmetric in-

teractions involving dozens of plant species and a wide variety of animal dispersers (Hollander & Vander Wall 2004; Thompson 2006; Meng *et al.* 2012). This makes such systems ideal for studying diffuse co-evolution between plants and animals. However, how functional traits contribute to the formation of such mutualisms is unknown. Furthermore, previous studies of dispersal networks have rarely quantitatively assessed assemblage structure, and have not considered predation simultaneously with mutualism. Consequently, there is a need to analyze network structure using quantitative interaction strength data (Bascompte *et al.* 2006).

In forest ecosystems, many nut-bearing trees depend on animals for seed dispersal. These plant species often employ a common strategy of producing highly nutritional seeds for attracting animals (Vander Wall 2001). Dispersed seeds often have a higher probability of seedling establishment due to improved microhabitat and reduced competition from the parent trees (Wenny 2001; O’Farrill *et al.* 2013). However, although animals benefit trees by dispersing seeds, they consume seeds and impose high predation pressure. Therefore, seeds have to evolve mechanisms to cope with the conflicting selection pressures and produce nutrient-rich seeds to attract dispersers and protections against overconsumption by animals. Understanding how seed traits affect the hoarding behavior of animals is essential for revealing the formation of mutualism–predation interactions between trees and animals (Guimaraes *et al.* 2011; Nuismer *et al.* 2013).

When rodents encounter seeds they usually face 2 decisions: eat them *in situ* or carry them to other places. Different behavioral decisions can often result in different outcomes for these plants. Seeds that have been scatter-hoarded in soil would be beneficial to seed dispersal and regeneration by reducing predation and desiccation and improving hydration and germination. In contrast, seeds that have been eaten or larder-hoarded in burrows will not contribute to plant recruitment (Beck & Vander Wall 2010; Hirsch *et al.* 2012). Seed dispersal is accomplished when these scatter-hoarded and unconsumed seeds germinate. Many seed traits have been identified to affect the hoarding behavior of rodents, including seed size (Vander Wall 2003; Jansen *et al.* 2004; Wang & Chen 2009), hardness of seed coat (Jacobs 1992; Zhang & Zhang 2008; Chang *et al.* 2012), nutrient content (Vander Wall 1995; Xiao *et al.* 2006b) and secondary compounds (Wang & Chen 2008; Xiao *et al.* 2008; Perea *et al.* 2011). However, these seed traits, through influencing the hoarding behavior of animals,

have not been quantitatively assessed in driving the formation of mutualism and predation interactions among multiple tree and animal species.

There are 3 main hypotheses to predict hoarding decisions by hoarding animals based on seed traits. First, the handling time hypothesis predicts that large seeds or seeds with a hard coat are more likely hoarded by animals because animals will require a longer handling time to eat them and, consequently, would suffer a high predation risk (Jacobs 1992; Jorge *et al.* 2012). Second, optimal foraging theory predicts that foragers should select food items that maximize net energy intake per unit time during the foraging process (Phelan & Baker 1992; Gerber *et al.* 2004). Accordingly, animals should hoard highly nutritional or high calorie seeds. Third, the high-tannin hypothesis predicts that hoarding animals prefer to eat low-tannin food items first but hoard high-tannin items for later consumption because tannins hinder food digestion by animals (Steele *et al.* 1993; Xiao *et al.* 2008). Testing these hypotheses would help us understand the occurrence of mutualism *versus* predation interactions between seeds and rodents.

In this study, we used semi-natural enclosures to investigate the hoarding behavior of 4 rodent species on the seeds of 8 tree species in a tropical forest of Yunnan, a province in Southwest China, and quantitatively assessed the relationship between seed traits and the hoarding behavior of rodents. The handling time hypothesis, the optimal foraging hypothesis and the high tannin hypothesis were tested. The network structures between seeds and rodents were constructed and the roles of seed traits in the formation of mutualism and predation interactions were tested. We aimed to explore the effects of seed traits and taxonomic groups on the occurrence of mutualism *versus* predation interactions as well as on the network structure in the seed–rodent system. We predict that taxonomically-related groups of tree species with similar seed traits should have similar mutualism *versus* predation interactions with rodent species, and determine the network structure of this seed–rodent dispersal system.

MATERIALS AND METHODS

Study site

We conducted our study in the Menglun Nature Reserve, Xishuangbanna, Yunnan, China. This region is dominated by a typical tropical monsoon climate with distinct rainy (May to Oct) and dry seasons (Nov to

Apr). The average annual temperature of this area is 21.8 °C. The annual precipitation varies from 1200 to 1700 mm, of which approximately 85% occurs during the rainy season. The dry season is characterized by heavy radiation fog, which supplements the deficiency of rainfall in this season (Cao & Zhang 1997).

Plant species

Our study site was close to the Xishuangbanna Tropical Rainforest Ecosystem Station (21°50'N, 101°12'E). This station was located in moist valley between 2 small hills. The valley and the bottom part of the hillside was typical tropical seasonal rainforest, and the upper part of the hillside was tropical evergreen broad-leaved forest. The former is dominated by trees of *Pometia tomentosa*, *Terminalia myriocarpa*, *Barringtonia macrostachya*, *Girardinia subaequalis*, *Ardisia tenera*; the evergreen trees are dominated by *Castanopsis echidnocarpa*, *Aporosa yunnanensis*, *Olea rosea*, *Lithocarpus truncates*, *Schima wallichii* and *Castanopsis calathiformis* (Zhang & Cao 1995). Acorns of 8 tree species in the family Fagaceae were studied: *Castanopsis echidnocarpa*, *Castanopsis calathiformis*, *Castanopsis hystrix*, *Castanopsis mekongensis*, *Lithocarpus leucostachyu*, *Lithocarpus truncatus*, *Lithocarpus magneinii* and *Quercus acutissima*. These species are either dominant or common species in the tropical evergreen broad-leaved forest, and sometimes appeared in the tropical seasonal rainforest (Zhu *et al.* 2006). Our previous and ongoing studies have confirmed that seeds of all these tree species in the 2 forest types are largely dispersed by small rodent species (Cao & Guo 2011).

Rodent species

In tropical forests of the study areas, Chinese white-bellied rats (*Niviventer confucianus* Milne-Edwards, 1871), chestnut rats (*Niviventer fulvescens* Gray, 1847), yellow-bellied rats (*Rattus flavipectus* Temminck, 1844) and red spiny rats (*Maxomys surifer* Miller, 1900) are the dominant rodent species, and are abundant. Field studies have indicated that these rodent species prefer to consume and hoard seeds of the family Fagaceae. Tree squirrels and birds are also potential seed dispersers, but they may be less important than the 4 rodent species because of their low abundance (Cao *et al.*, unpubl. data).

We selected 3 plots to investigate the relative abundance of small rodents in different seasons in our study sites from Dec 2008 to Jun 2011. Plot 1 and 2 were tropical evergreen broad-leaved forest, and plot 3 was a tropical seasonal rainforest. Each plot covers an area of

500–600 m² and the distances between them were 500–1000 m. To trap rodents, we used large wired cages (30 × 14 × 14 cm) baited with shelled peanuts. We set out 50 traps, 10 m apart along 5 transect lines in each plot. The traps were checked each dawn for 3 consecutive days. The species, sex and body weight of captured rodents were recorded, and they were released immediately *in situ*.

The target rodents used in the enclosure experiments were captured from other stands approximately 500 m away from the above 3 plots. Adult rodents in a non-reproductive state were transferred to our laboratory and housed individually in plastic boxes (40 × 30 × 25 cm) with adequate commercial mouse chow, water and nest structures. The housing room was maintained at 18–25 °C with a natural photoperiod cycle. All the subjects were housed for at least 2 weeks in order for them to habituate to the laboratory environment. A total of 20 adult *N. confucianus* (11 ♂, 9 ♀, mean body weight 86 ± 11 g), 20 adult *N. fulvescens* (8 ♂, 12 ♀, mean body weight 69 ± 9 g), 14 adult *R. flavipectus* (7 ♂, 7 ♀, mean body weight 127 ± 13 g) and 14 adult *M. surifer* (8 ♂, 6 ♀, mean body weight 115 ± 14 g) were used in this experiment. Animals were handled according to protocols approved by the Institute of Zoology, Chinese Academy of Sciences. After the experiments were finished, all rodents were released where they were captured.

Seeds collection and preparation

Eight sympatric acorn species (mentioned above) were selected for this study because they are very common in our study site and have overlapping seed rain periods between Sep and Nov. We collected fresh target seeds from the ground after they were mature, and used a water floating method to detect insect-damaged and empty seeds, which were discarded. Then we randomly selected 100 undamaged acorns of each species for measuring morphological traits in our laboratory (Table 1). A mixture of dry cleaned seed kernels (30–50 g) of each tree species was provided to use as samples for measuring nutritional traits. Seed nutrient composition, crude protein, crude fat, crude starch and tannins were tested by the Cereal Quality Supervision and Testing Centre, Ministry of Agriculture, China. The caloric value of each seed species (kJ/g) was estimated by the average gross energy equivalents of protein (17.2 kJ/g), starch (17.2 kJ/g) and fat (38.9 kJ/g) (Yang & Xiao 2002), and the caloric value per seed of each seed species (kJ) using mean dry kernel mass multiplied by the caloric value.

Table 1 Morphological (mean \pm SE, $N = 100$) and nutritional traits of the 8 seed species

Seed species	Fresh weight (g)	Coat thickness (mm)	Crude protein (%)	Crude fat (%)	Crude starch (%)	Tannins (%)	Calories per seed (kJ)
<i>C. echidnocarpa</i>	0.45 \pm 0.01	0.43 \pm 0.01	3.8	0.4	73.5	0.15	2.82
<i>C. calathiformis</i>	0.72 \pm 0.03	0.42 \pm 0.01	4.5	0.24	51.17	6.22	3.29
<i>C. hystrix</i>	0.93 \pm 0.04	0.34 \pm 0.01	3.08	0.25	75.86	0.15	6.15
<i>C. mekongensis</i>	3.31 \pm 0.04	1.07 \pm 0.01	6.75	0.13	66.28	0.17	16.01
<i>L. leucostachyus</i>	1.08 \pm 0.02	0.90 \pm 0.01	4.37	0.58	61.81	1.99	4.18
<i>L. truncatus</i>	1.26 \pm 0.03	1.02 \pm 0.01	4.38	0.6	61.16	2.19	5.17
<i>L. magneinii</i>	2.20 \pm 0.04	0.79 \pm 0.01	3.74	0.13	68.3	1.44	12.32
<i>Q. acutissima</i>	3.18 \pm 0.11	0.62 \pm 0.01	3.42	3.13	NA	10.7	NA

NA, no data (a result of some technical problems).

Seeds used in this experiment were marked by attaching to a numbered plastic tag (2.5 \times 3.6 cm, 0.25 g) through a metal wire (8 cm long, 0.5 mm diameter) to facilitate relocation. This tagging method has been proved to have a negligible effect on seed removal and hoarding by rodents and to be effective in tracing seeds (Xiao *et al.* 2006a).

Enclosure design

Four 10 \times 10 m semi-natural enclosures were established in an artificial rubber plantation. The enclosures were built with concrete and bricks approximately 1.5 m high and extending 0.5 m below ground. Wire mesh supported by steel frames was installed on the top of the enclosures to prevent focal rodents from escaping or other animals from entering. Pieces of plastic cloth were covered above the wire mesh to prevent rainwater from getting into the enclosures during the experiments. To simulate the natural vegetation of the study area, many shrubs and herbaceous plants were artificially transplanted inside the enclosures (approximately 25 m²). For each enclosure, a nest box (40 \times 40 \times 40 cm) with some leaves buried in the ground was used as an artificial burrow. A tilted PVC pipe (15 cm diameter, 40–50 cm long) connected the underground burrow to the ground surface, allowing rodents to enter or leave the artificial burrow freely.

Experimental procedure

One week before the experiments, we fed the subjects with seeds of 8 acorn species so they could become familiar with them. Subjects were also introduced

into the enclosure for 1 night to become acclimatized to the testing environment. Each trial lasted 5 days. To initiate a trial, on the first day at 1730 hours, we placed 80 marked seeds (10 seeds of each species) in the center of the enclosure, and then released a subject into the enclosure. In each of the following 4 days at 0830 hours, we searched the enclosure thoroughly (including the underground burrow) and recorded the fate of each seed. All predated seeds were removed and intact seeds were kept at their original location. In addition, we recorded the spatial coordinates of each cache of buried seeds within the enclosure. After 4 days, the subject and all remaining seeds or their fragments were taken out of the enclosure and the experiment was ended.

Seed fates were recorded as follows: ‘remained intact *in situ*’ (RIS) when the tagged seeds remained intact at the release site; ‘eaten’ (E) when the tagged seeds were consumed completely or partially and the tag or fragments left on the ground or in the burrow; ‘scatter-hoarded’ (SH) when the tagged seeds were intact and buried in the enclosure; and ‘larder-hoarded’ (LH) when the tagged seeds were moved into the burrow and well preserved. RIS represents seed not harvested (removed or eaten) by rodents. E represents seed eating during hoarding. SH represents seed caching outside of nests. LH represents seed caching in nests. We define SH as representing a mutualism between rodents and the tree species, while E+LH represent seed predation because eaten or larder-hoarded seeds do not produce seedlings. Because RIS contains the combined impact of E, SH and LH, we do not analyze the RIS results.

Statistical analyses

Using the group average method, we performed a cluster analysis to classify the 8 experimental acorns based on the seed traits of these tree species. All data (seed traits) were standardized with a range from 0 to 1. In our experiment, each subject stayed in the enclosure for 4 days; we recorded the seed fates every day. For data analyses, we only used the final seed fates, which measured by proportion of seeds handled by rodents in all 4 days (accumulative data). Because seed traits are highly correlated with each other, we used Spearman's correlation coefficients to explore the effects of each seed trait on the final seed fates. Cluster analysis was also used to identify the substructure of mutualism and predation networks between seeds and rodents based on the interaction strength for each final seed fate. Interaction strength was also measured by proportion of seeds handled by rodents in all 4 days.

RESULTS

Seed traits and seed classification

Seeds of the 8 experimental species showed large variation in their traits (Table 1). Cluster analysis grouped the 8 acorn species into 4 clusters, representing 4 types (Fig. 1). Cluster 1 contains 3 species of genus *Castanopsis* (*C. echidnocarpa*, *C. calathiformis* and *C. hystrix*), which have low fresh seed weights (also small

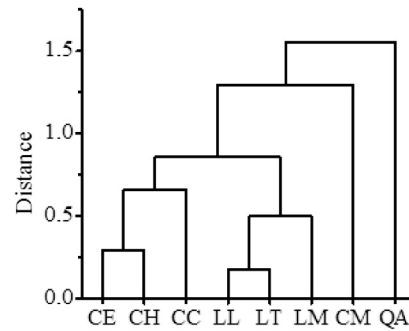


Figure 1 Cluster analysis for 8 acorn species based on seed traits for which data on all 8 acorns were available (excluding crude starch and calories per seed). CE, *C. echidnocarpa*; CC, *C. calathiformis*; CH, *C. hystrix*; LL, *L. leucostachyus*; LT, *L. truncates*; LM, *L. magneinii*; QA, *Q. acutissima*; CM, *C. mekongensis*.

size) and thin seed coats. Within this group, *C. calathiformis* has the highest tannin content. Group 2 contains 3 *Lithocarpus* species (*L. leucostachyus*, *L. truncates* and *L. magneinii*), which have medium seed weights and seed coat thickness. Within this group, *L. magneinii* has the highest calories per seed. Group 3 only consists of *C. mekongensis*, which has the highest seed weight, coat thickness, crude protein content and calories per seed. Group 4 consists of *Q. acutissima*, which has the highest tannin and crude fat content.

Table 2 Population abundance and compositions of rodent species in study area (3 plots data combined together)

Survey time	Traps [†]	<i>N. confucianus</i>	<i>N. fulvescens</i>	<i>R. flavipectus</i>	<i>M. surifer</i>	<i>L. edwardsi</i>	Total	Trap success (%)
Dec 2008	450	5	1	0	0	1	7	1.56
Mar 2009	450	11	2	0	0	0	13	2.89
Sep 2009	450	2	0	0	2	0	4	0.89
Dec 2009	450	7	0	0	1	0	8	1.78
Mar 2010	450	10	2	1	2	0	15	3.33
Jun 2010	450	14	6	6	5	1	32	7.11
Sep 2010	450	13	2	0	3	0	18	4.00
Dec 2010	450	10	3	1	1	2	17	3.78
Mar 2011	450	8	1	1	1	0	11	2.44
Jun 2011	450	11	0	0	0	0	11	2.44
Total	4500	91	17	9	15	4	136	3.02
Proportion (%)		66.91	12.50	6.62	11.03	2.94		

[†]Traps: 50 traps/plot/day, 3 consecutive days, 3 plots.

Rodent species and population abundance

Over 3 years, we captured 136 individual rodents belonging to 5 species (Table 2). *N. confucianus* was the most common species and comprised 66.9% of all captures. The other 4 species, *N. fulvescens*, *R. flavipectus*, *M. surifer* and *L. edwardsi*, were less abundant and comprised 12.5%, 6.62%, 11.03% and 2.94% of all captures, respectively. The trap success in 2010 was considerably higher than those in 2009 and 2011 (Table 2).

Effects of seed traits on hoarding behaviors

As shown in Table 3, the proportion of seeds eaten (E) by *N. confucianus* was negatively correlated with both fresh seed weight and seed coat thickness; the proportions of seeds eaten by *N. fulvescens* and *M. surife* were only negatively correlated with fresh seed weight; the proportion of seeds eaten by *R. flavipectus* was negatively correlated with tannins.

The proportion of seeds scatter-hoarded (SH) by *N. confucianus* were positively correlated with fresh seed weight and seed coat thickness; the proportion of seeds scatter-hoarded by *M. surife* was positively correlated with fresh seed weight, seed coat thickness and calories per seed.

The proportion of seeds larder-hoarded (LH) by both *N. confucianus* and *N. fulvescens* were positively correlated with fresh seed weight, seed coat thickness and calories per seed, respectively; the proportion of seeds larder-hoarded by *M. surife* were positively correlated with both fresh seed weight and seed coat thickness; the proportion of seeds larder-hoarded by *R. flavipectus* were only positively correlated with seed fresh weight.

The proportion of seeds predation (E+LH) by *M. surife* was negatively correlated with fresh seed weight; the proportion of seeds predation by *N. confucianus* was positively correlated with crude starch but negatively correlated with tannins.

Seed-rodent network structure

For seeds eaten by rodents, cluster analysis indicated that acorns of the 8 species were grouped into 2 clusters. Cluster 1 contains seeds of *C. echidnocarpa*, *C. calathiformis* and *C. hystrix*. Cluster 2 contains the other 5 species. Seeds of cluster 1 are small seeds with soft coats, while cluster 2 are medium or large sized with hard coats. Cluster 2 seeds were eaten less by rodents than those of cluster 1 due to the large size and the hard coats of the seeds (Table 3, Figs 2a and 3a). For seeds eaten by rodents, 4 rodent species were grouped into 2

Table 3 Spearman's rank correlations between seed traits and the proportion of seeds handled by sympatric rodent species

Rodent species	Seed fate	Fresh weight	Coat thickness	Crude protein	Crude fat	Crude starch	Tannin	Calories per seed
<i>N. confucianus</i>	E	-0.81*	-0.71*	-0.29	-0.3	0.5	-0.59	-0.43
<i>N. fulvescens</i>	E	-0.71*	-0.45	0.14	-0.28	0.36	-0.66	-0.46
<i>M. surifer</i>	E	-0.74*	-0.48	0.12	-0.3	0.39	-0.68	-0.5
<i>R. flavipectus</i>	E	-0.54	-0.35	0.12	-0.25	0.49	-0.72*	-0.22
<i>N. confucianus</i>	SH	0.76*	0.87**	0.55	-0.38	-0.34	0.19	0.72
<i>N. fulvescens</i>	SH	0.5	0.71	0.46	-0.47	-0.14	-0.19	0.75
<i>M. surifer</i>	SH	0.86**	0.83**	0.31	-0.3	-0.11	0.07	0.89**
<i>R. flavipectus</i>	SH	No scatter-hoarding						
<i>N. confucianus</i>	LH	0.92**	0.84**	0.27	-0.02	-0.13	0.18	0.81*
<i>N. fulvescens</i>	LH	0.83*	0.90**	0.35	-0.15	-0.13	0.05	0.81*
<i>M. surifer</i>	LH	0.84**	0.77*	0.19	-0.09	-0.05	0.1	0.74
<i>R. flavipectus</i>	LH	0.77*	0.5	-0.06	0.16	-0.31	0.59	0.51
<i>N. confucianus</i>	E+LH	-0.29	-0.24	-0.14	-0.52	0.86*	-0.92**	0.25
<i>N. fulvescens</i>	E+LH	-0.7	-0.47	0.12	-0.33	0.4	-0.68	-0.41
<i>M. surifer</i>	E+LH	-0.74*	-0.48	0.12	-0.3	0.39	-0.68	-0.5
<i>R. flavipectus</i>	E+LH	-0.46	-0.24	0.08	-0.07	0.52	-0.69	-0.14

*Correlation was significant at the 0.05 level. **Correlation was significant at the 0.01 level. E, eaten; LH, larder-hoarding; E+LH, representing predation; SH, scatter-hoarding.

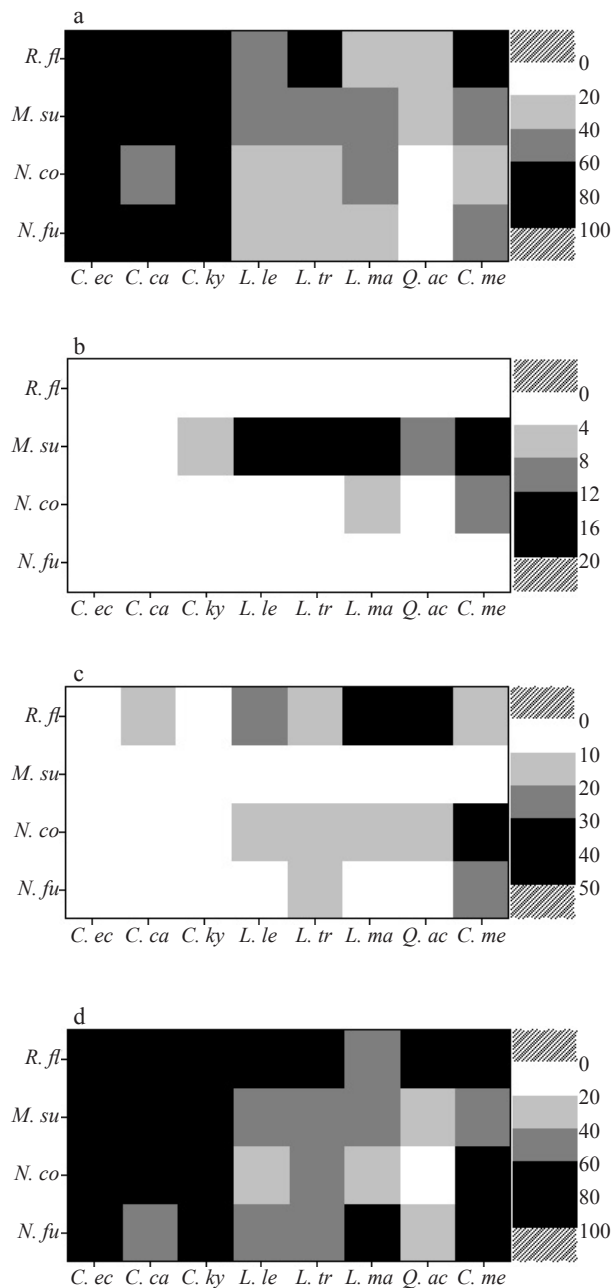


Figure 2 Interaction strength between seeds and rodents for each seed fate as measured by proportion of seeds handled by rodents (%), and represented by the darkness. Eaten (a), scatter-hoarding (mutualism) (b), larder-hoarding (c), predation (d). *C. ec*, *C. echidnocarpa*; *C. ca*, *C. calathiformis*; *C. hy*, *C. hystrix*; *L. le*, *L. leucostachyus*; *L. tr*, *L. truncatus*; *L. ma*, *L. magneinii*; *Q. ac*, *Q. acutissima*; *C. me*, *C. mekongensis*; *N. co*, *N. confucianus*; *N. fu*, *N. fulvescens*; *R. fl*, *R. flavipectus*; *M. su*, *M. surifer*.

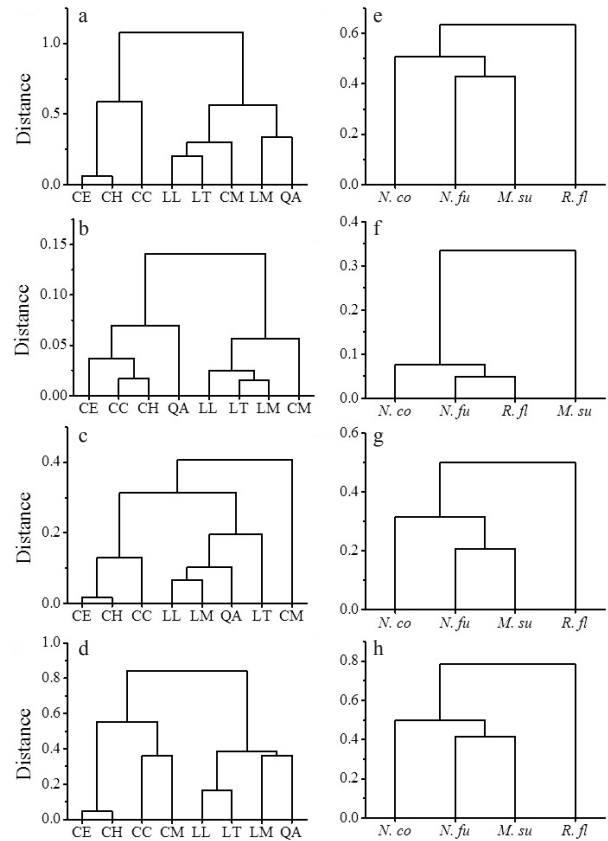


Figure 3 Cluster analysis for seeds (left) and rodents (right) based on interaction strength for each seed fate. Eaten (a, e), scatter-hoarding (b, f), larder-hoarding (c, g), predation (d, h). Abbreviations: CE, *C. echidnocarpa*; CC, *C. calathiformis*; CH, *C. hystrix*; LL, *L. leucostachyus*; LT, *L. truncatus*; LM, *L. magneinii*; QA, *Q. acutissima*; CM, *C. mekongensis*; *N. co*, *N. confucianus*; *N. fu*, *N. fulvescens*; *R. fl*, *R. flavipectus*; *M. su*, *M. surifer*.

clusters: cluster 1 contains *N. confucianus*, *N. fulvescens* and *M. surifer* and cluster 2 contains *R. flavipectus* only. *R. flavipectus* of cluster 2 has the largest body size, and tends to eat more hard-coated seeds (e.g. *L. leucostachyus*, *L. truncatus* and *C. mekongensis*) (Figs 2a and 3e).

For seeds scatter-hoarded by rodents, cluster analysis indicated that acorns of the 8 species were grouped into 2 clusters: cluster 1 contains seeds *C. echidnocarpa*, *C. calathiformis*, *C. hystrix* and *Q. acutissima*; and cluster 2 contains the other 4 species. Seeds of cluster 2 generally have large seed size and a hard seed coat; they were more frequently scatter-hoarded than cluster 1 seeds (Table 3, Figs 2b and 3b). For seeds scatter-hoarded by rodents, 4 rodent species were grouped into 2 clusters.

Cluster 1 contains *N. confucianus*, *N. fulvescens* and *R. flavipectus*. Cluster 2 contains *M. surifer* only. *M. surifer* of cluster 2 were typically scatter-hoarders, and they scatter-hoarded many more seeds than the other rodent species (Figs 2b and 3f).

For seeds that were larder-hoarded by rodents, cluster analysis indicated that the acorns of the 8 species were grouped into 3 clusters. Cluster 1 contains seeds *C. echidnocarpa*, *C. calathiformis* and *C. hystrix*, which have small seed size and thin coats; cluster 3 only contains seed of *C. mekongensis*, which has the largest seed size, a hard seed coat and the greatest calories per seed; and cluster 2 contains the other 4 species. Seeds of cluster 3 were mostly larder-hoarded, while seeds of cluster 2 and 3 were also highly larder-hoarded due to their large fresh weight, high coat thickness and high calories per seed (Table 3, Figs 2c and 3c). For seeds that were larder-hoarded by rodents, 4 rodent species were grouped into 2 clusters. Cluster 1 contains *N. confucianus*, *N. fulvescens* and *M. surifer*. Cluster 2 contains *R. flavipectus* only, which did not scatter-hoard any seeds; they larder-hoarded many seeds, especially more seeds with high tannin content (e.g. *C. calathiformis* and *Q. acutissima*) than the other 3 rodent species did (Figs 2c and 3g).

For predation interactions, cluster analysis indicated that the acorns of the 8 species could be grouped into 2 clusters: cluster 1 contains seeds of 4 *Castanopsis* species (*C. echidnocarpa*, *C. calathiformis*, *C. hystrix* and *C. mekongensis*); and cluster 2 contains the other 4 species (Table 3, Figs 2d and 3d). For predation interactions, 4 rodent species were grouped into 2 clusters: cluster 1 contains *N. confucianus*, *N. fulvescens* and *M. surifer*; and cluster 2 contains *R. flavipectus* only. *R. flavipectus* shows predominant negative effects on seeds (it is a pure seed-eater), as compared to the other 3 rodent species (Figs 2d and 3h).

DISCUSSION

In this study, by using semi-natural enclosures, we quantitatively assessed the mutualism and predation interactions between each seed and rodent species. We found that seed traits and taxonomically-related group are important in shaping the seed–rodent dispersal network structure. Seeds of 3 tree species (*C. echidnocarpa*, *C. calathiformis* and *C. hystrix*) have small seed weight, soft seed coats and low calories per seed; they showed frequent predation but low mutualism interactions with rodent species. Seeds of other 3 tree species,

L. leucostachyus, *L. truncatus* and *L. magneinii*, have medium seed weight and hard seed coats; they showed infrequent predation but frequent mutualistic interactions with rodent species. Seeds of *Q. acutissima* have high tannin content, showed infrequent predation and mutualistic interactions with rodent species. Seeds of *C. mekongensis* have the highest seed weight, hardest seed coat and highest calories per seed; they showed frequent predation and mutualistic interactions with rodent species.

Our quantitative data on interaction strength revealed clear substructures of mutualism and predation interactions between seeds and rodents. It is interesting that the 2 clusters of tree species observed in the seed–rodent dispersal system are represented by 2 genera, *Castanopsis* (except for *C. mekongensis*) and *Lithocarpus*. The observed clusters of tree species based on interaction with rodents correspond well to the clustering results of the 8 tree species based on seed traits, suggesting that taxonomically-related species generally possess similar seed traits with respect to dispersal and seed predation. Such species would then form similar clusters in the assemblages of seeds and rodents as functional groups. We did not find consistent and clear clusters for rodents, but there were large variations among the rodent species. *M. surifer* showed frequent mutualistic interactions with seeds. *N. confucianus* and *N. fulvescens* showed infrequent to moderately frequent mutualistic interactions with seeds. *R. flavipectus* as a pure seed-eater showed no mutualism with seeds.

Various previous studies have demonstrated that rodents are inclined to remove and hoard large seeds but eat small seeds *in situ*, indicating seed size plays an important role in seed fate (Xiao *et al.* 2005; Zhang *et al.* 2008). In this study, seed weight showed consistent negative effects on the proportion of seeds eaten by 3 rodent species, a positive effect on the proportion of seeds scatter-hoarded by 2 rodent species, and a positive effect on the proportion of seeds larder-hoarded by all 4 rodent species. A commonly hypothesized explanation is that with the increase in seed size, the handling time by rodents would increase, and this would bring high predation risk for rodents eating seeds rather than hoarding them (Jorge *et al.* 2012). Our results support this handling time hypothesis. Thus, this difficult handling trait would be beneficial to the formation of mutualisms. However, difficult handling trait also increases larder-hoarding, which may explain the observation that *C. mekongensis*, with the heaviest seed weight and hardest seed coat, has high predation as well as high

mutualism. In addition, larger or heavier seeds generally contain more energy (caloric value) and, according to optimal foraging theory (Krebs 1977), rodents should forage for these seeds, which best maximize their net energy intake for a limited time. This is especially critical for many rodents when they can carry only 1 seed at a time. The food value of small seeds might not warrant a large investment of time to hoard, so small seeds are more often eaten *in situ* or moved only short distances.

Seeds of many tree species that are adapted for dispersal by seed-hoarding animals have hard coats (e.g. a woody endocarp). Removing a hard coat not only increases the energy expenditure, but also increases the handling time, thereby increasing the individual's predation risk. Hence, this physical barrier could influence the likelihood of seed-hoarding rodents to hoard or eat. Previous studies have found that seeds with hard coats are eaten less *in situ* but are hoarded more (Xiao *et al.* 2006b; Zhang & Zhang 2008). Our results indicate that coat thickness tended to reduce seed predation by 1 rodent species; tended to increase seeds scatter-hoarding by 2 rodent species and seed larder-hoarding by 3 rodent species (also supporting the handling time hypothesis).

Nutrient content is an important investment for seed germination and early plant establishment, meanwhile play a significant role in the seed foraging strategies of rodents (Vander Wall 2001). Seeds with higher concentration of nutrients are more attractive and beneficial to rodents, and stimulate hoarding behavior (Vander Wall 1995; Xiao *et al.* 2006b). In this study, we found that calories per seed tended to increase seed scatter-hoarding by 1 rodent species and larder-hoarding by 2 rodent species. These results support the nutrition hypothesis as predicted by the optimal foraging theory whereby animals prefer to hoard highly nutritional seeds to maximize net energy income. This is complicated by the fact that small rodents that scatter-hoard seeds in soil are known to retrieve the seeds by means of spatial memory; however, other rodents can also rely on olfaction or random search to pilfer seeds (Dally *et al.* 2006). Seeds with high nutritional value are more attractive to rodents and may be pilfered more than other seeds. Thus, highly nutritional seeds would have high pilferage risk due to their strong odor, and then would be more likely to be larder-hoarded by rodents, which may weaken the mutualistic effect of the high nutrition trait.

Tannins, a large group of polyphenolic compounds that are commonly found among plant seeds, are believed to be chemical defenses used to discourage some

seed predators through the incursion of metabolic costs to them after the seeds have been eaten (Chung-MacCoubrey *et al.* 1997; Skopec *et al.* 2004). With the increase of tannins, the metabolic costs imposed on rodents would increase because of detoxification requirements. Thus tannins decrease the immediate value of seeds, influencing the feeding and hoarding decision of rodents. According to the high-tannin hypothesis, small rodents would prefer to consume more seeds with low-tannin levels immediately but hoard more seeds with high-tannin levels for later consumption. Many studies have provided strong evidence to support this hypothesis (Steele *et al.* 1993; Wang & Chen 2008; Xiao *et al.* 2008). In our study, tannin content had a positive effect on the proportion of seed that remained intact at the release site by 3 rodent species, and a negative effect on the proportion of seed eaten by 1 species, showing the defensive effect of tannins on rodents. However, we did not see the significant positive effect of tannins on hoarding, possibly because the variation in tannins is not large enough within the seeds we studied. These results partially support the toxicant hypothesis. The presence of tannins can effectively reduce the seed digestion of rodents because of their negative health effects. Thus, seeds with high tannin content would be cached for later use because they still have potential value when other food is scarce.

Seed dispersal by scatter-hoarding rodents is crucial for many large-seeded plant species (Briggs *et al.* 2009; Hirsch *et al.* 2012). Although rodents consume large proportions of seed crops of the plant, they also have important positive impacts on seedling establishment and plant regeneration by caching some seeds in the soil (Gomez *et al.* 2008). However, in most situations, seed dispersal occurs through the combined effect of a seed-eating guild (Hollander & Vander Wall 2004). These animal species can be found consuming seeds of many plant species (negative effect), and provide their dispersal services through scatter-hoarding behavior (positive effect). Our study demonstrated that the 4 sympatric rodent species in a tropical forest showed clear differentiation in seed consumption and hoarding behavior, thus play different roles in plant regeneration. *M. surifer* scatter-hoarded many seeds, larder-hoarded few seeds and were the dominant dispersers in our study area compared to the other 3 rodent species. While *N. confucianus* and *N. fulvescens* larder-hoarded many seeds, they scatter-hoarded some seeds, so they were also important seed dispersers. *R. flavipectus* did not scatter-hoard any seeds, but larder-hoarded large amount of seeds,

so they had no beneficial effect on these plants. Our results also demonstrate that seeds with small mass, soft seed coat and low calories per seed (*C. echidnocarpa*, *C. calathiformis* and *C. hystrix*) were less scatter-hoarded by rodents. However, tree species with small seeds often produce large amount of seeds, which may ensure the survival of a certain number of seeds within an area, reflecting a different strategy of trees with small seeds from those with medium or larger seeds.

It is notable that our results are achieved in the enclosure conditions without the presence of potential competitors or predators of rodent species, and variation of seed fall in both time and space. All of these factors may affect or modify the observed interactions among seeds and rodents. Future studies should use novel approaches in the quantitative assessment of interactions among multiple species in natural conditions.

In summary, by using quantitative data on interaction strength, we found that there are clusters of species in the seed–rodent dispersal system that are represented by 2 genera in the family Fagaceae, which own similar seed traits. Seed traits and taxonomic relation may play significant roles in shaping mutualism and predation interactions as well as the structure of the assemblage of rodents and tree species in our study system.

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