Plant Ecol (2016) 217:253–265
DOI 10.1007/s11258-016-0566-0

Trade-off between seed defensive traits and impacts on interaction patterns between seeds and rodents in forest ecosystems

**Zhibin Zhang · Zhenyu Wang · Gang Chang · Xianfeng Yi ·
Jiqi Lu · Zhishu Xiao · Hongmao Zhang · Lin Cao ·
Fusheng Wang · Hongjun Li · Chuan Yan**

Received: 3 August 2015 / Accepted: 11 January 2016 / Published online: 21 January 2016

Abstract Plants often have two kinds of defensive traits against animal predation: physical and chemical defenses, but the trade-off between them is heavily debated, and their impacts on relationship between plants and animals are largely unknown. We investigated seed traits of 23 tree species and their impacts on seed fates or hoarding behaviors under predation from 16 rodent species in four forest types in China. We provide clear evidence that there is a strong nonlinear trade-off between physical (as measured by seed coat thickness) and chemical (as measured by tannin content) defensive traits in seeds. This trade-off was closely associated with nutritional traits, resulting in coordinated defense syndromes in seeds. The seed fate and hoarding behavior patterns were largely determined by the trade-off-related seed traits and the body mass of rodents, respectively, not by the phylogenetic relations of species. Tree species showed more conservative evolution in seed traits of high starch content, high tannin content, and thin seed coat, but they showed more convergent/divergent evolution in seed traits of high protein content, high fat content, and thick seed coat under rodent predation. Our results suggest that trade-off-related seed traits may play a predominant role in shaping the relationship between plants and animals.

Keywords Trade-off · Physical and chemical defense · Phylogenetic distance · Evolution · Plant-animal interactions

---

Electronic supplementary material The online version of this article (doi:10.1007/s11258-016-0566-0) contains supplementary material, which is available to authorized users.

Z. Zhang (✉) · Z. Wang · Z. Xiao · F. Wang ·
H. Li · C. Yan
State Key Laboratory of Integrated Management on Pest
Insect and Rodent in Agriculture, Institute of Zoology,
Chinese Academy of Sciences, 1 Beichen Xilu,
Chaoyang District, Beijing 100101, China
e-mail: zhangzb@ioz.ac.cn

Z. Wang · X. Yi
College of Life Sciences, Jiangxi Normal University,
Nanchang 330022, China

G. Chang
Shanxi Institute of Zoology, Xi'an 710032, China

J. Lu
Institute of Biodiversity and Ecology, Zhengzhou
University, Zhengzhou 450001, China

H. Zhang
Molecular and Behavioural Research Group, College of
Life Sciences, Central China Normal University,
Wuhan 430079, China

L. Cao
Center for Integrative Conservation, Xishuangbanna
Tropical Botanical Garden, Chinese Academy of
Sciences, Mengla 666303, Yunnan, China

Introduction

Trade-off arising from physiology and allocation is a fundamental feature of organisms in nature (Stearns 1989; Leishman 2001; Zera and Harshman 2001). Because plant defense is energy consuming, it is predicted that there should be trade-offs between resource investments in different types of anti-herbivore defenses (Lebreton 1982; Steward and Keeler 1988; Karban and Myers 1989; Herms and Mattson 1992; Koricheva et al. 2004; Kempel et al. 2011). However, the defense trade-off theory is still heavily debated. Some studies indicated that there were significant and negative correlations between chemical and physical defense in leaves of plants (Twigg and Socha 1996; Hanley and Lamont 2002), but others did not support such observation (Koricheva et al. 2004; Read et al. 2009). Some authors argued that seeds might form coordinated defense syndromes (Agrawal and Fishbein 2006). A recent study indicated that there was marginally significant correlation between species' overall chemical and physical defense levels which did not support arguments for trade-offs or for coordinated defense syndromes (Moles et al. 2013).

Seeds are important foods for animals in forest ecosystems, and they often suffer strong predation pressure from animals like insects, mammals, and birds (Xiao et al. 2002; Carlo and Yang 2011; Chang and Zhang 2014). Previous studies have demonstrated that there are two main seed defense strategies, in response to animal attack, involving physical and chemical defenses (Xiao et al. 2008; Zhang and Zhang 2008). The chemical defense traits of seeds include mainly tannins, while physical defense traits of seeds include stingers, the seed coat, endocarp, and so on. According to the defense trade-off theory, there should be a trade-off between physical and chemical defense in seeds for countering predation by animals (e.g., Lebreton 1982; Steward and Keeler 1988). There are three basic kinds of nutrition in seeds: protein, fat, and carbohydrate (e.g., starch) (Chang and Zhang 2014). It is likely that different kinds of nutrition in seeds may differ in their association with defensive traits. Previous studies indicated that tannin was not incompatible with protein (Adamczyk et al. 2012), which may result in selection of defense-related nutrition association or coordinated defense syndromes. Although trade-offs between physical and chemical defenses have been investigated in leaves of many plant species for countering herbivores (Twigg and Socha 1996; Hanley and Lamont 2002; Read et al. 2009), such studies in seeds under animal predation are still lacking, especially across a large range of taxa and regions. It is still unknown how trade-off between defensive traits and associated nutrition traits affect seed fates under rodent predation.

The functional traits of organisms are often determined by two factors: adaptation to environmental conditions and by shared ancestry (Powell et al. 2009). Convergent evolution occurs if similar traits are observed in species from different lineages, while divergent evolution occurs if closely related species evolve different traits (Gulick 1888; Reece et al. 2010). Conservative evolution occurs if functional traits were largely invariant within descendent lineages (trait conservation) as compared to variation among lineages. Therefore, phylogenetic relations would significantly influence the convergent and divergent evolution in seed traits under animal predation and, therefore, the ecological interaction patterns between plants and animals. Recently, the role of evolutionary signals in affecting species interaction networks have been well investigated (e.g., Bascompte and Jordano 2014; Rohr and Bascompte 2014). Impacts of evolutionary signals on ecological interaction pattern between plants and animals would be large if conservative evolution prevails, otherwise, it would be small if convergent/divergent evolution prevails. In general, links between evolutionary and ecological networks through functional traits (particularly trade-off-related seed traits) are rarely investigated.

The purpose of this study was to investigate the trade-off between defensive traits (physical defense vs chemical defense) and the impacts of trade-off-related traits (e.g., protein, fat, and starch) on seed fates or hoarding behaviors across a broad range of taxa and geographical regions. Seed fates or hoarding behaviors of rodents were measured based on tests of hoarding behaviors of 125 animals from 16 rodent species on seeds of 23 tree species in semi-natural enclosures in the temperate, warm temperate, subtropical, and tropical forest ecosystems in China. Seed fate clustering patterns of trees or hoarding behaviors clustering patterns of rodents represent ecological interaction patterns between tree and rodent species. Seed trait clustering pattern of trees represents the evolutionary pattern of trees in seed traits under rodent predation.

We had three objectives. First, we wanted to test the defensive trade-off hypotheses and the coordinated defense syndrome hypothesis in seeds. We predicted that there would be a negative association between physical (as measured by seed coat thickness) and chemical (as measured by tannin contents) defensive traits, and nutrition traits might be closely associated with these trade-off traits. Second, we wanted to examine the impacts of the seed traits on seed fates and hoarding behaviors of rodents to infer the role of trade-off-related traits. Finally, we wanted to examine associations among clustering patterns of phylogenetic distance, seed traits, and seed fates or hoarding behaviors, so as to infer the impacts of trade-off-related traits on relationship between seeds and rodents in forest ecosystems.

Methods

Study sites

We conducted studies in four research stations located in the Dailing (45°58′ N, 129°08′ E, elevation 750 m), Donglingshan (40°00′N, 115°30′E, elevation 1140 m), Dujiangyan (31°4′N, 103°43′E, elevation 700–1000 m), and Xishuangbanna (21°56′N, 101°15′E, elevation 550 m) stations in China during 2004–2011 (Supplementary Materials, Fig. S1). The Dailing station is located in the temperate broad-leaved deciduous forest in northeast China. The Donglingshan station is located in the warm-temperature broad-leaved deciduous forest in north China. The Dujiangyan station is located in the subtropical evergreen broad-leaved deciduous forest in south China. The Xishuangbanna station belongs to the subtropical rainforest in southwest China. For more detailed information about the four study sites, see Cheng et al. (2005) and Chang and Zhang (2014) for the Dujiangyan station; Li and Zhang (2003, 2007) and Zhang and Zhang (2008) for the Donglingshan station; Yi et al. (2008) and Yi and Zhang (2008) for the Dailing station; and Cao et al. (2011) and Wang et al. (2014) for the Xishuangbanna station.

Seed collection and seed traits

Seeds were collected when they became mature using seed traps (Xiao et al. 2001). The seed traps were made of nylon net bags (mesh size 2 mm × 2 mm) with a diameter about 0.8 m at the open entry (supported by a wire with a diameter of 5 mm) and placed under trees with support of three wooden sticks about 1.2 m high above the ground (the distance of the bag bottom to the ground was about 0.6 m). Mature seeds were collected mainly from August to October in the Dailing site, from July to September in the Donglingshan site, from September to November in the Dujiangyan site, and from August to December in the Xishuangbanna site. Intact seeds without infestation of fungus or damages by animals (e.g., insect, mammals, and birds) were selected and kept in cool and dry environmental conditions before enclosure tests. We randomly selected some intact seeds of each tree species to measure their seed traits (Table S1; for more details, see refs. Zhang and Zhang 2008; Chang and Zhang 2014; Wang et al. 2014). A mixture of dry seed kernels of each tree species was used to measure their nutritional traits (including crude protein, crude fat, crude starch, tannin, and caloric value per seed) in the Cereal Quality Supervision and Testing Centre, Ministry of Agriculture, China. The seed tannin content was measured according to the national standard (GB/T15686-2008) for sorghum using the spectrophotometry method. The seed protein content was measured according to the national standard (NY/T3-1982) for cereals and legumes using the semi-micro Kjeldahl apparatus. The seed fat content was measured according to the national standard (NY/T4-1982) for cereals and oil crops using the Soxhlet extractor. The seed starch content of dry nutmeat was measured according to the national standard (NY/T11-1985) for cereals using the optical rotation method. The caloric values of seeds per gram (KJ/g) were calculated by the average gross energy equivalents of protein (17.2 kJ/g), fat (38.9 kJ/g), and carbohydrates (17.2 kJ/g) (Yang and Xiao 2002); the caloric value per seed (KJ) was calculated as mean dry kernel mass per seed × caloric value per gram. The seed coat thickness was used to represent the physical defense trait of seeds, while the tannin content was used to represent the chemical defense trait. We used the vernier caliper to measure the minimum thickness of the seed coat. We defined thick- or thin-coated seeds based on the relative thickness (large or small) of their seed coat. Because animals often prefer to eat high protein or/and fat foods (Li and Liu 2003; Makarios-Lahham et al. 2004; Wang and Chen 2009;

Zweifel-Schielly et al. 2012), we defined protein and/or fat as the high-nutrient food and starch as low nutrient food for rodents. The taxonomic trees of tree and rodent species in our study were shown in the supplementary materials (Figs. S2 and S3).

Rodent trapping

We captured rodents in the field nearby the study plots using live traps made of steel wire mesh (L × W × H = 14 × 14 × 30 cm) and baited with fresh peanuts (Chang et al. 2009). The species, body mass, and reproductive status of captured animals were recorded (Table S2). Pregnant females or juveniles were immediately released in situ. Before enclosure tests, all captured animals were kept individually in cages at the field laboratory and provided with adequate food, water, and nest materials for daily use. Procedures of capturing and raising animals were in accordance with the regulations by the Institute of Zoology, Chinese Academy of Sciences.

Hoarding behaviors and seed fates in enclosures

We built several semi-natural enclosures (standard size for each enclosure: L × W × H = 10 × 10 × 1.5 m) in each of the four study sites by following Cheng et al. (2005). To keep rodents in the enclosures and exclude other large animals from the enclosures, we built enclosure walls using concrete, with the wall above ground 1-m high and 0.5-m deep underground. The top of the enclosure was covered with wire netting of a small mesh size (1.5 × 1.5 cm). In the south sites of Dujiangyan and Xishuangbanna, the top of the enclosure was covered with plastic cloth to prevent disturbance from rainfall during the experiment. Modifications in construction of enclosures were needed in different sites to adapt the experiment to local environments. Nest boxes were placed in each corner of the enclosure, and one food station (supplied with water) was placed in the center of the enclosure for rodents.

The experiments on seed fates and hoarding behaviors of rodents lasted for 2 days (minor modifications of the following experimental procedures were needed to adapt the experiment to local environments). Only one animal was placed in each enclosure and provided with seeds of several tree species of the study site (for tree species used in each study site, see Table S1). The animal was used only once for each enclosure test. A total of 125 animals of 16 rodent species (shown in Table S2) were used to study seed fates of 23 tree species (shown in Table S1). In the first day, one animal was placed in the enclosure and provided with tagged seeds to allow the animal to adapt to the new environment. On the second day, 10 seeds of each plant species were placed in the food stations to test the impacts of seed traits on seed fates by rodents. Seeds were tagged with light plastic-coded tags (3 × 1 cm) attached using 10 cm of thin (diameter 0.2 mm) stainless-steel wire (Zhang and Wang 2000; Xiao et al. 2006a, b). This seed tagging method has been shown to have a negligible effect on seed removal and hoarding (Xiao et al. 2006a, b). After seed placement, seed fates were examined next day.

Following our previous studies, seed fates were defined as follows:

IIS: Intact seeds left at seed stations (i.e., intact in situ) which represents the intensity of seed removal by rodents.

EIS: Seeds consumed by rodents at seed stations (i.e., eaten in situ).

EAR: Seeds removed and consumed by rodents away from seed stations (i.e., eaten after removal).

IAR: Intact seeds dropped on ground surfaces away from stations (i.e., intact after removal).

SH: Intact seeds cached in soil or leaf litter (i.e., scatter hoarded) which represents a mutualism between seeds and rodents if seeds later germinate.

LH: Intact seeds preserved in the nest boxes (i.e., larder hoarded).

These indices of seed fates affect dispersal success of seeds in early seed dispersal stage (Zhang et al. 2008). After seed fates were examined, all seed fragments were cleared to prepare for the next day's test.

For each animal j of a rodent species i, the percent ($p_{i,j,k}$, %) of each seed fate (i.e., IIS, EIS, EAR, IAR, SH, LH) of a tree species k was calculated as number of seeds of the focal seed fate/total number of seeds of the focal tree species placed in the enclosure × 100 %. For a given rodent species i, the average percent (p_0, %) of the focal seed fate of the focal tree species k was calculated as

$$p_{i,k} = \sum_{j=1}^{n} \frac{p_{i,j,k}}{n}$$

($j = 1$ to n; n is the number of animals of the rodent species i tested, which represents replicates). We used the average percent (p_0, %) for further statistical analysis.

Statistical analysis

Proportional data were arcsine transformed before statistical analysis for achieving or approaching a normal distribution. Kolmogorov-Smirnova and Shapiro–Wilk tests were used to explore the data normality. Data were naturally logarithm-transformed to detect potential nonlinear relations. Pearson (for normally distributed data) or Spearman (for not normally distributed data) correlation was used to identify significant correlations among variables of seed traits, seed fates, and body mass of rodents. Factor analysis was used to identify key principle components in determining seed traits of all tree species by following Chang and Zhang (2014). SPSS statistics (version 20) were used for theses statistical analysis.

General linear mixed models (GLMM) were used to test the effects of seed traits and body mass of rodents with sites being taken as a random factor. The best-fitting models were selected by the lowest AIC values from 256 candidate models (all possible combinations of variables) for each response variable. The taxonomic trees for tree and rodent species (Figs. S2 and S3) in our study were used by referring to the Catalogue of Life China 2013 Annual Checklist (http://www.sp2000.cn/joaen/).

Square Euclidean distance (standardized as z scores) was calculated to infer similarity of seed traits or seed fates (or hoarding behaviors) between pairwise species of trees or rodents, and to identify significant clusters ($p < 0.05$) so as to infer evolutionary or ecological patterns of tree species by comparing the differences among clustering patterns of phylogenetic distance, seed traits, seed fates, or hoarding behaviors. By searching GenBank (http://www.ncbi.nlm.nih.gov/genbank/), we only found 16 tree species of our study had the gene sequence data, and only one gene of about 200 bp was available for establishing the phylogenetic trees. The phylogenetic tree based on this short gene sequence is not reliable. Thus, we used the online Phylomatic service (http://www.phylodiversity.net/phylomatic) and PhyloCom software to construct a phylogenetic tree for tree species (Webb et al. 2008). The phylogenetic tree for rodents was constructed by cutting the species from a worldwide species-level mammalian supertree with branch lengths (calculated from multiple genes) (Fritz et al. 2009), using picante package in R software (R Core Team 2014). Phylogenetic distance (PD), which represents the phylogenetic relatedness between pairwise species of tree or rodents, was calculated from the branch lengths of the two phylogenetic trees, using ape package in R software (R Core Team 2014).

The evolutionary adaptation of seed traits or seed fates to rodent predation was defined as convergent, divergent, and conservative evolution. If species from different taxonomic clades (i.e., genera) appeared in the same seed trait or seed fate clades, we defined this phenomenon as the convergent evolution. If species from the same taxonomic clade (i.e., genus) appeared in different seed trait or seed fate clades, we defined it as the divergent evolution. If species from same taxonomic clade (i.e., genus) appeared in the same seed trait or seed fate clade, we defined it as the conservative evolution.

Mantel test, which is widely used for comparing distance matrices in ecology (Goslee and Urban 2007), was used to test the correlation between phylogenetic distance, seed trait distance, and seed fate distance in tree species and also the correlation between phylogenetic distance and hoarding behavior distance in rodent species. If the correlation between phylogenetic distance and seed trait distance or seed fate distance was significant and positive, we concluded that the phylogenetic relatedness played a significant role in affecting seed traits or seed fates, and conservative evolution in seed traits or seed fates was recognized; otherwise, convergent or divergent evolution were recognized. R software (R Core Team 2014) was used for the statistical analysis of general linear mixed models and Mantel test.

Results

Trade-off between defensive traits

Correlation analysis indicated that the physical defense trait of (seed coat thickness) was negatively correlated with the chemical defense trait of tannin content ($p < 0.05$; Figs. 1, 2a; Table S3), and this negative and nonlinear relationship was very strong (with a fitting curve: ln (coat thickness) = −0.250–0.163 ln (tannin content), $p = 0.042$) (Fig. 2a). High nutrition trait (protein) was positively associated with the physical defensive trait (seed coat thickness, $p < 0.05$) but negatively associated with chemical defense of tannin content ($p < 0.05$) (Fig. 1, Table S3), while low nutrition trait (starch content) was negatively associated with seed coat thickness, protein, and fat contents (all $p < 0.001$).

Seed weight was positively associated with the coat thickness ($p < 0.01$) and the caloric values ($p < 0.01$). The caloric value was positively associated with fat content ($p < 0.01$) (Fig. 1, Table S3). Similar to results of Fig. 1 and Table S3, factor analyses also revealed that coat thickness, fat and protein content, seed weight, and caloric value were positively associated with each other, but they were negatively associated with starch and tannin contents (Supplementary Materials, Fig. S4)

Hence, these analyses indicated that there was obvious nonlinear trade-off between physical and chemical defense traits, and physical defense was associated with high nutritional traits, while chemical defense was associated with low nutrition traits (with an approximate significance level, $p < 0.1$).

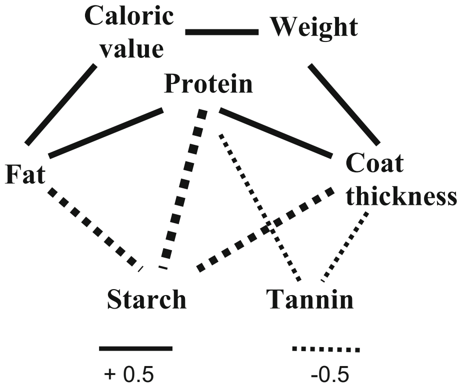

Fig. 1 Significant correlations among seed traits. *Solid lines* denote positive correlations and *dashed lines* denote negative correlations. The *thickness of the lines* denotes values of Pearson or Spearman correlation coefficients as contrasted to that of *lines* (correlation coefficient is +0.5 for *solid lines* and −0.5 for *dashed lines*) in the key below the figure

Effects of trade-off-related seed traits

Spearman correlation analyses indicated that percent of seeds intact in situ (IIS) was positively correlated with seed weight and coat thickness (all $p < 0.001$), but negatively related to starch content (all $p < 0.01$) (Table S4). Percent of seeds eaten in situ (EIS) was positively correlated with starch content ($p < 0.01$) but negatively correlated with seed weight, coat thickness, protein content (all $p < 0.001$), fat content ($p < 0.01$), and caloric value ($p < 0.05$). Percent of eaten after removal (EAR) was negatively correlated with seed weight ($p < 0.01$) and coat thickness ($p < 0.001$). Percent of seeds larder hoarded (LH) was positively correlated with seed weight ($p < 0.05$) and coat thickness ($p < 0.001$) but negatively correlated with tannin content ($p < 0.05$). Body mass of rodents was negatively correlated to IIS ($p < 0.01$).

GLMM analyses indicates that coat thickness showed a positive effect on IIS, but a negative effect on EIS and EAR; body mass of rodents (see Table S2) showed a positive effect on EIS; fat content showed a negative effect on EAR (Table 1).

Hence, these analyses indicated that seeds with large seed weight or thick coat or high nutritional or high caloric value were less frequently eaten but more frequently larder hoarded, while low nutritional or high tannin seeds were more frequently eaten but less frequently larder hoarded. Large-sized rodents harvested more seeds.

Effects of phylogenetic signals

The phylogenetic tree of tree species indicated that a large group of species belonged to Fagaceae, and the other species showed high coat thickness except for *P. kerrii* and *C. oleifera* (Fig. 3a, b). The Fagaceae group was characterized by high starch content (Chang et al. 2012; Table S1). Within the Fagaceae group, there were three genera: *Lithocarpus*, *Castanopsis*, and *Quercus*. Seeds of *Lithocarpus* had very thick seed coat, while *Castanopsis* and *Quercus* had very thin seed coat (some of them had high tannin content). Cluster analyses based on seed traits of tree species (Fig. 3a, b) showed significant associations between phylogenetic distance and seed trait distance (Mantel test: r = 0.39, $p < 0.05$). There were three significant seed trait clades of tree species (Fig. 3b). The first clade (# 1) was composed of three species (*J.*

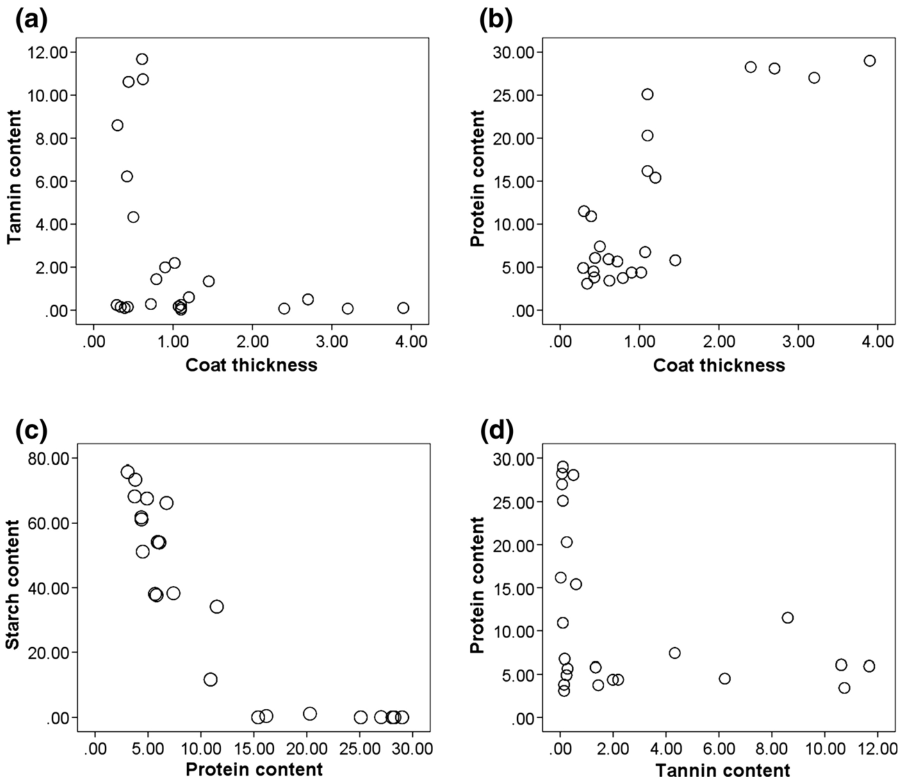

Fig. 2 Correlations between defensive and nutritional seed traits of 23 tree species. **a** negative correlation between coat thickness and tannin content, **b** positive correlation between coat thickness and protein content, **c** negative correlation between protein content and starch content, and **d** negative correlation between tannin content and protein content

Table 1 The best-fitting models selected by lowest AIC values from 256 candidate models for each response variable

Best-fitting models	AIC
IIS = 0.275 coat thickness	111.44
EIS = 0.0014 body mass − 0.1213 coat thickness	44.96
EAR = −0.1839 coat thickness − 0.0058 fat content	34.73

Seed fates were defined as seeds intact in situ (IIS), eaten in situ (EIS), eaten after removal (EAR), intact after removal (IAR), scatter hoarded (SH), and larder hoarded (LH)

mandshurica, A. davidiana, J. regia) with high nutrition and very thick seed coat. The second clade (# 2) was composed of species (i.e., *P. koraiensis, C. mandshurica, A. vulgaris, C. oleifera, and C. heterophylla*) with high nutrition (high fat and protein contents) and thick seed coat (except for *C. oleifera*). The third, also the largest clade (# 3), was mostly composed of species of Fagaceae group (except for *P. kerrii*) which was characterized by high starch content. Within this largest clade (# 3), there were three non-significant sub-clades, and one of them was composed of high tannin species (i.e., *Q. variabilis, Q. serrata, C. calathiformis, Q. wutaishanicaa, and Q. mongolica*). The third clade (# 3) corresponded well to their phylogenetic relations, showing strong conservative evolution in seed traits of starch content (Fagaceae clade) and tannin content (*Quercus* cluster), but the other two clades (# 1, # 2) did not correspond well with their phylogenetic relations, showing strong convergent evolution in seed traits of seed coat thickness and high nutrition. However, convergent evolution was also observed in a few species within the Fagaceae clade. *Castanopsis* genus was characterized with very thin seed coat and low tannin content, but *C. menkongensis* (with thick seed coat) and *C. calathiformis* (with high tannin content) showed divergent evolution from their genera in seed trait clades.

Cluster analyses based on seed fates revealed five significant clades (Fig. 3c). The first and the largest clade (# 1) were characterized with thick seed coat for most tree species (except for *P. kerrii* and *C. oleifera*), or with high nutrition (except for *P. kerrii*). The second (# 2), third (# 3), and fifth (# 5) clade were composed of tree species with thin seed coat or/and high tannin contents. The fourth clade (# 4) was composed of only one species with thick seed coat and high nutrition. It is notable that all species of *Lithocarpus* with relatively thick seed coat within

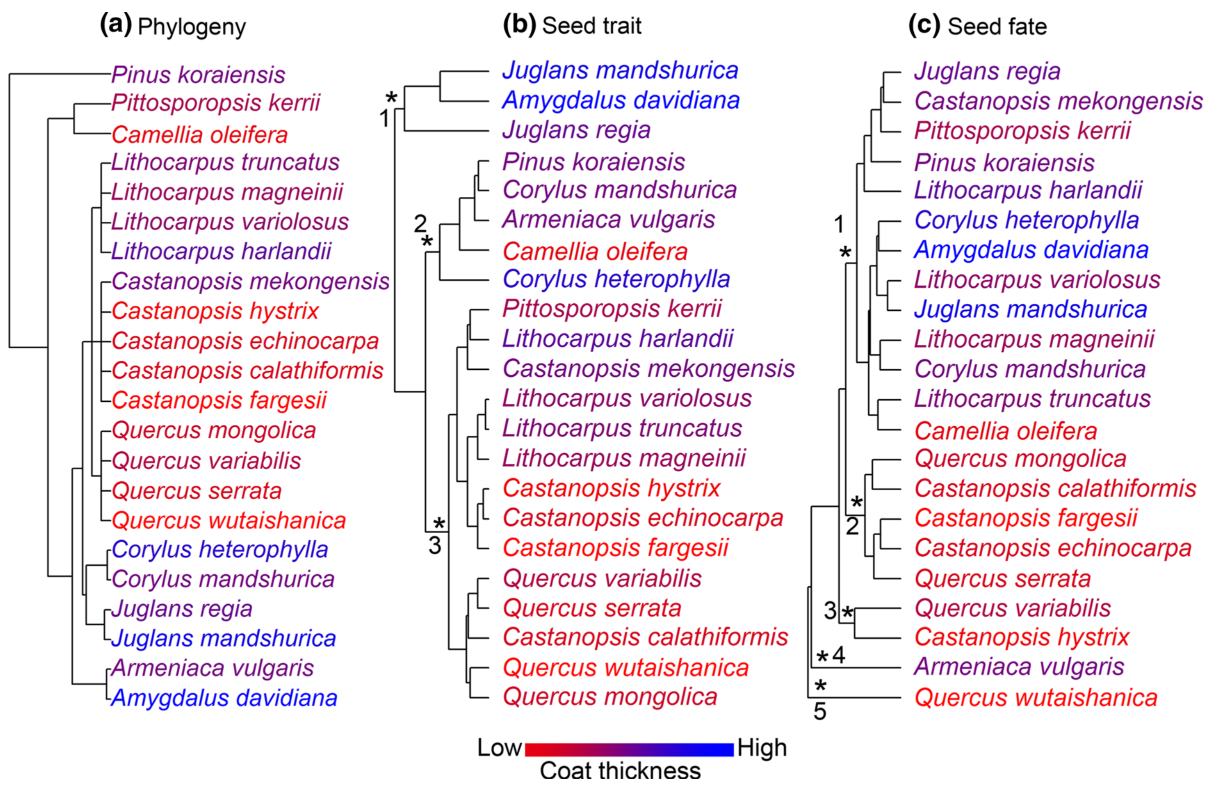

Fig. 3 Effects of phylogenetic relation on seed trait and seed fate patterns of tree species under rodent predation. Phylogeny of rodent species (**a**), seed trait clades (**b**), and seed fate clades (**c**). *Asterisk* denote significant clusters, numbers indicate cluster code, the color from *red* to *blue* indicates the coat thickness of seeds from soft to hard

the Fagaceae clade were grouped into the first seed fate clade (# 1). All species with thick seed coat, high nutrition and low tannin were grouped into the first seed fate clade (# 1) except for one species (*A. vulgaris*), and all species in this clade had thick seed coat and high nutrition except for one species (*P. kerrii*). All species with thin seed coat, low nutrition or/and high tannin contents were grouped into the other four groups except for *A. vulgaris*. Mantel test indicated that the pairwise phylogenetic distance of tree species was not significantly correlated to the square Euclidean distance of seed fates ($p > 0.05$).

The phylogenetic reconstruction of rodents indicated that the largest clade was composed of species of Murinae; within this Murinae group, *Apodemus* was characterized by small body mass (Fig. S4, Fig. 4a). Cluster analyses based on hoarding behaviors of rodents (corresponding to seed fates of tree species) revealed four significant hoarding behavior clades (Fig. 4b). The largest cluster (# 1) was characterized by small body mass of rodents, while the other three clusters (# 2, # 3, # 4) were characterized by large body mass. Mantel test showed the phylogenetic distance was not significantly correlated to the square Euclidean distance of hoarding behaviors ($p > 0.05$)

Hence, these results indicated that trade-off in seed traits or rodent body mass were more associated with ecological interaction patterns among rodents and seeds, but the effects of phylogenetic distance of tree or rodents were not significant.

Discussion

Trade-off between defensive seed traits

On the basis of physiological and ecological costs of defense allocation, most plant defense hypotheses predict the occurrence of trade-off between resource

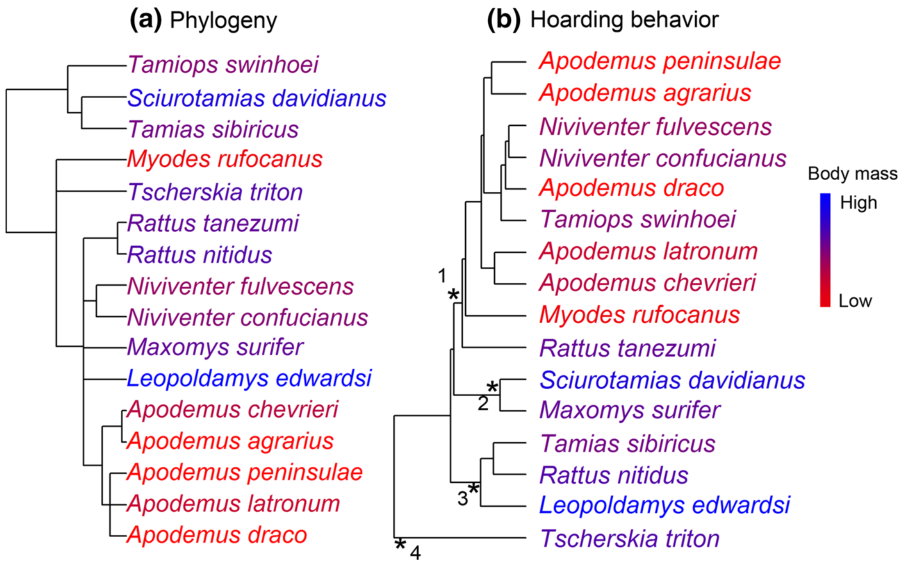

Fig. 4 Effects of phylogenetic relation on hoarding behavior patterns of rodent species. Phylogeny of rodent species (**a**), hoarding behavior clades of rodents (**b**). *Asterisk* denote significant clusters, numbers indicate cluster code, the color from *red* to *blue* indicates the body mass of rodents from small to large

investments in different types of anti-herbivore defenses (Koricheva et al. 2004). The defensive trade-off hypothesis was supported in several studies on leaves of plants (e.g., Twigg and Socha 1996; Hanley and Lamont 2002), not supported by others (e.g., Read et al. 2009; Koricheva et al. 2004; Moles et al. 2013). Our results provided the first evidence of nonlinear trade-off between physical and chemical defense in seeds for countering animal predation across a large range of taxa and regions. Seed coat thickness is significantly and negatively correlated with the tannin content in seeds of 23 tree species from four climate zones, supporting the defensive trade-off hypothesis. In our study, seeds countered rodent predation with a spectrum of two defensive strategies, physical defense or chemical defense. Thick seed coat or endocarp has been shown to effectively reduce the speed of seed destruction, and significantly increase the time for rodents to eat seeds (Zhang and Zhang 2008). Similarly tannin compounds are often toxic to rodents and can effectively slow down seed consumption by rodents (Xiao et al. 2008). Both physical and chemical defenses are costly in energy, and thus it is logical that seeds show trade-off between these two defensive traits. The two defensive strategies are less compatible with each other, resulting in strong nonlinear negative relations between seed coat thickness and tannin content (Fig. 2a).

We also found the nutritional traits were closely associated with the two different defensive traits. Protein and fat content were positively correlated with seed coat thickness, but negatively correlated with tannin and starch contents. Starch content was positively correlated with tannin content (with an approximate significant level) but negatively correlated with seed coat thickness, protein and fat contents. High tannin seeds frequently also have high starch content and low protein/fat content (e.g., *Q. serrata*, *Q. variabilis*, and *Q wutaishanica*; Table S1). Tannins can precipitate proteins, which inhibits the absorption of nutrients in some ruminant animals (Adamczyk et al. 2012). Indeed, we also found tannin and protein are not compatible with each other in seeds; their relation was a “L” shape (Fig. 2d). A large intake of tannins may cause irritation of bowel, stomach or kidney, liver damage, or gastrointestinal pain (Adamczyk et al. 2012). The incompatibility between tannin and protein may explain why tannin is more associated with starch content, and less correlated with protein or fat content. Our results show that physical defense is selected for protecting high nutrition seeds (with high protein or fat), while tannin is likely selected for protecting low nutrition seeds with high starch. We suggest that the incompatibility between protein and tannin may play a central role in the evolution of defense and nutrition traits in seeds. Furthermore, we found seed mass was closely associated with seed coat thickness and high protein or fat content, supporting the hypothesis of coordinated defense syndromes in plants (Agrawal and Fishbein 2006; Moles et al. 2013).

Effects of trade-off-related seed traits

Several hypotheses have been suggested to explain the effects of seed traits on seed fates under rodent predation, but most studies are conducted based on one location or a few species. The high tannin hypothesis suggests that high tannin seeds are more likely dispersed by rodents, not consumed in situ (Steele et al. 1993; HadjChikh et al. 1996; Wang and Chen 2008; Xiao et al. 2008, 2009) because tannin is known to inhibit food digestion of rodents (Smallwood and Peters 1986). The handling time hypothesis predicts that large seeds or seeds with hard endocarp are more likely to be hoarded by animals because of the longer handling time required to eat them and consequently the animals would suffer a high predation risk (HadjChikh et al. 1996; Xiao et al. 2005; Yi and Zhang 2008). High fat seeds are seen to be more hoarded by rodents for storage (Xiao et al. 2006a, b), while low nutrition seeds (e.g., insect-infested seeds) are more likely eaten in situ, less hoarded by rodents. Our results indicate that seed traits are highly correlated with the trade-off-related traits (Fig. 1). Seed traits of large seed weight, thick seed coat, high protein, and fat contents were positively associated together. Likewise, seed traits of high tannin content and high starch content were often positively associated. The seed fates were not affected by a single trait, but a combined effect of coordinated defense syndromes (Agrawal and Fishbein 2006). We found that thick coat and low tannin seeds with high protein and fat significantly not only reduced seed consumption and predation by rodents, but also reduced seed removal. We also found high tannin and high starch seeds not only suffered high seed consumption and predation by rodents, but increased seed removal. Our results using data from a broad range of taxa and regions generally suggest that the seed fate clustering patterns of tree species are mainly determined by the trade-off-related traits. It is notable that thickness of seed coat does not necessarily mean the hardness. Thus, it is necessary to establish the relationship between seed coat thickness and hardness in future studies.

The effects of body size of rodents on seed fates have rarely been investigated, probably due to a limited number of rodent species being studied in one location. Rodents with different body sizes may have different seed-handling abilities and, therefore, have different seed selection pressure on seed size (Lu and Zhang 2005, 2007, 2008). It has been reported that large rodents can consume both small and large seeds, while small rodents only select small seeds (Zhang and Zhang 2008; Izhaki 2002; Munoz and Bonal 2008). Chang and Zhang (2014) suggested that in the Dujiangyan site, large-sized rats tend to scatter hoard more large and thick coat seeds, while small-sized mice tend to scatter hoarding high tannin seeds. Our results indicated that large rodent species tended to harvest more seeds and ate seeds in situ, and thus imposed high predation pressure on seeds. This is likely because large animals are more capable of countering predation risk of their predators and needs more food for maintenance per individual. It is notable that the hoarding behavior patterns of rodents were mainly determined by body mass of rodents (Fig. 4b), suggesting body size of animals is very important in shaping the seed-rodent interactions.

Effects of the phylogenetic signals

Previous studies demonstrated that phylogenetic relationships contributed to the formation of community structure and diversity (Silvertown et al. 2001, Webb et al. 2002, Rezende et al. 2007) or species interaction network (Bascompte and Jordano 2014; Rohr and Bascompte 2014), but it is still not clear how they are linked to functional traits (particularly the trade-off-related traits). Closely related species of trees may have similar seed traits and then have similar seed fates under animal predation (a tendency of conservative evolution). But under natural selection of similar environment (e.g., seed predation or dispersal), distantly related species may show similar seed fates (a tendency of convergent evolution). Our results indicated that under rodent predation pressure, seed fates were more influenced by the trade-off-related seed traits (i.e., seed coat thickness and nutrition quality) (Fig. 3c). The influence of phylogenetic relation is minor, although in two genera (*Castanopsis* and *Quercus*) show similar seed fates. For majority of non-Fagaceae group, tree species showed obvious convergent evolution in seed traits of high physical defensive trait (thick seed coat) and high nutrition traits (protein and fat), while for majority of Fagaceae group, tree species showed obvious conservative evolution in low nutrition trait (starch), high chemical defensive trait (tannin), and low physical defensive trait (thin seed coat) (Fig. 3b). Within the Fagaceae group, divergent evolution in seed traits toward medium-thick seed coat occurred in a few species (four *Lithocarpus* species and *C. mekongnsis*). It is obvious that trade-off in defensive seed traits has weakened the effects of phylogenetic signals on seed fate patterns of tree species. However, seed fate clades #2 and #3 are species mostly from two genera of *Castanopsis* and *Quercus*, suggesting some influence of phylogenetic signals in limited groups. Similarly, we also found the hoarding behavior pattern was mainly determined by the body size of rodents, not by their phylogenetic relations (Fig. 4b). However, rodent species within the Murinae group (Fig. S3, Fig. 4a) had small body size (except for *L. edwardsi*) and had similar hoarding behavior patterns (Fig. 4b), suggesting potential impact of phylogenetic relations in this group. Chang and Zhang (2014) found that small-sized *Apodemus* species tend to hoard small and high tannin seeds in the Dujiangyan region.

In summary, we found there was a clear evidence of nonlinear trade-off between physical and chemical traits in seeds of tree species over a broad range of taxa and regions. This trade-off resulted in coordinated defense syndrome in seeds. The trade-off-related traits are closely associated with the seed trait and seed fate patterns of trees under rodent predation. Our results suggested trade-off might play a significant role in shaping evolutionary and ecological patterns of species interactions in animal-plant systems. Future studies should be directed to investigate effects of trade-off in seed traits on seedling establishment in natural conditions.

Acknowledgments This work is partially supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDB11050300), the grants of National Natural Science Foundation of China (30930016, 31330013, 31372212), the Knowledge Innovation Program of Chinese Academy of Sciences (KSZD-EW-TZ-008; CXTDS2005-4), and the National Basic Research Program of China (2007CB109101; 2012CB955504). We are grateful to Prof. Marcel Holyoak, University of California, for his valuable comments to this manuscript. We thank Mr. Yunfa Xiao and Xunlong Wang for the field assistances in rodent capture in Dujiangyan region.

References

Adamczyk B, Salminen JP, Smolander A, Kitunen V (2012) Precipitation of proteins by tannins: effects of concentration, protein/tannin ratio and pH. Int J Food Sci Technol 47:875–878

Agrawal AA, Fishbein M (2006) Plant defense syndromes. Ecology 87:S132–S149

Bascompte J, Jordano P (2014) Mutualistic networks. In: Leven SO, Horn HS (eds) Monographs in population biology, vol 53. Princeton University Press, Princeton

Cao L, Xiao ZS, Wang ZY, Guo C, Chen J, Zhang ZB (2011) High regeneration capacity helps tropical seeds to counter rodent predation. Oecologia 166:997–1007

Carlo TA, Yang S (2011) Network models of frugivory and seed dispersal: challenges and opportunities. Acta Oecol 37:619–624

Chang G, Xiao ZS, Zhang ZB (2009) Hoarding decisions by Edward's long-tailed rats (*Leopoldamys edwardsi*) and South China field mice (*Apodemus draco*): The responses to seed size and germination schedule in acorns. Behav Process 82:7–11

Chang G, Zhang Z (2014) Functional traits determine formation of mutualism and predation interactions in seed-rodent dispersal system of a subtropical forest. Acta Oecol 55:43–50

Chang G, Jin T, Pei J, Chen X, Zhang B, Shi Z (2012) Seed dispersal of three sympatric oak species by forest rodents in the Qinling Mountains, Central China. Plant Ecol 213:1633–1642

Cheng JR, Xiao ZS, Zhang ZB (2005) Seed consumption and caching on seeds of three sympatric tree species by four sympatric rodent species in a subtropical forest, China. For Ecol Manag 216:331–341

Fritz SA, Bininda-Emonds ORP, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. Ecol Lett 12:538–549

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. J Stat Softw 22(7):1–19

Gulick JT (1888) Divergent evolution through cumulative segregation. J Linn Soc Lond Zool 20(120):189–274

HadjChikh LZ, Steele MA, Smallwood PD (1996) Caching decisions by grey squirrels: a test of the handling time and perishability hypotheses. Anim Behav 52:941–948

Hanley ME, Lamont BB (2002) Relationships between physical and chemical attributes of congeneric seedlings: how important is seedling defence? Funct Ecol 16:216–222

Herms DA, Mattson WJ (1992) The dilemma of plants—to grow or defend. Q Rev Biol 67:283–335

Izhaki I (2002) The role of fruit traits in determining fruit removal in East Mediterranean ecosystems. In: Levey DJ, Silva WR, Galetti M (eds) Seed dispersal and frugivory: ecology, evolution, and conservation. CABI Publishing, Wallingford, pp 161–175

Karban R, Myers JH (1989) Induced plant-responses to herbivory. Annu Rev Ecol Syst 20:331–348

Kempel A, Schadler M, Chrobock T, Fischer M, van Kleunen M (2011) Tradeoffs associated with constitutive and induced plant resistance against herbivory. Proc Natl Acad Sci USA 108:5685–5689

Koricheva J, Nykanen H, Gianoli E (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? Am Nat 163:E64–E75

Lebreton P (1982) Tanins ou alcaloïdes: deux tactiques phytochimiques de dissuasion des herbivores. Rev Ecol (Terre Vie) 36:539–572

Leishman MR (2001) Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. Oikos 93:294–302

Li HJ, Zhang ZB (2003) Effect of rodents on acorn dispersal and survival of the Liaodong oak (*Quercus liaotungensis* Koidz.). For Ecol Manage 176:387–396

Li HJ, Zhang ZB (2007) Effects of mast seeding and rodent abundance on seed predation and dispersal by rodents in *Prunus armeniaca* (Rosaceae). For Ecol Manag 242:511–517

Li J, Liu J (2003) Ecological implication and behavior mechanism of food selection of mammalian herbivores. Chin J Appl Ecol 14:439–442

Lu JQ, Zhang ZB (2005) Food hoarding behaviour of large field mouse *Apodemus peninsulae*. Acta Theriol 50:51–58

Lu JQ, Zhang ZB (2007) Hoarding of walnuts by David's rock squirrels (*Sciurotamias davidianus*) within enclosure. Acta Theriol Sin 27:209–214

Lu JQ, Zhang ZB (2008) Differentiation in seed hoarding among three sympatric rodent species in a warm temperate forest. Integr Zool 3:134–142

Makarios-Lahham L, Roseau SM, Fromentin G, Tome D, Even PC (2004) Rats free to select between pure protein and a fat-carbohydrate mix ingest high-protein mixed meals during the dark period and protein meals during the light period. J Nutr 134:618–624

Moles AT, Peco B, Wallis IR et al (2013) Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? New Phytol 198:252–263

Munoz A, Bonal R (2008) Are you strong enough to carry that seed? Seed size/body size ratios influence seed choices by rodents. Anim Behav 76:709–715

Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proc R Soc B 276:4237–4245

R Core Team (2014). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

Read J, Sanson GD, Caldwell E et al (2009) Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. Ann Bot 103:757–767

Reece JB, Campbell NA, Urry LA et al (2010) Cambell biology, vol 9. Pearson, San Francisco

Rezende EL, Lavabre JE, Guimaraes PR, Jordano P, Bascompte J (2007) Non-random coextinctions in phylogenetically structured mutualistic networks. Nature 448:925–926

Rohr RP, Bascompte J (2014) Components of phylogenetic signal in antagonistic and mutualistic networks. Am Nat 184(5):556–564

Silvertown J, Dodd M, Gowing D (2001) Phylogeny and the niche structure of meadow plant communities. J Ecol 89:428–435

Smallwood PD, Peters WD (1986) Gray squirrel food preferences: the effects of tannin and fat concentration. Ecology 67:168–174

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Steele MA, Knowles T, Bridle K, Simms EL (1993) Tannins and partial consumption of acorns: implications for dispersal of oaks by seed predators. Am Midl Nat 130:229–238

Steward J, Keeler KH (1988) Are there trade-offs among antiherbivore defences in Ipomoea (Convolvulaceae)? Oikos 53:79–86

Twigg LE, Socha LV (1996) Physical versus chemical defence mechanisms in toxic Gastrolobium. Oecologia 108:21–28

Wang B, Chen J (2008) Tannin concentration enhances seed caching by scatter-hoarding rodents: An experiment using artificial 'seeds'. Acta Oecol 34:379–385

Wang B, Chen J (2009) Seed size, more than nutrient or tannin content, affects seed caching behavior of a common genus of Old World rodents. Ecology 90:3023–3032

Wang ZY, Cao L, Zhang ZB (2014) Seed traits and taxonomic relationships determine the occurrence of mutualisms versus seed predation in a tropical forest rodent and seed dispersal system. Integr Zool 9:309–319

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098–2100

Xiao ZS, Wang YS, Zhang ZB (2001) Seed bank and the factors influencing it for three Fagaceae species in Dujiangyan Region, Sichuan. Biodivers Sci 9:373–381 **(In Chinese with English abstract)**

Xiao ZS, Zhang Z, Wang Y (2002) Rodent's ability to discriminate weevil-infested acorns: potential effects on regeneration of nut-bearing plants. Acta Theriol Sin 23:312–320

Xiao ZS, Chang G, Zhang ZB (2008) Testing the high-tannin hypothesis with scatter-hoarding rodents: experimental and field evidence. Anim Behav 75:1235–1241

Xiao ZS, Gao X, Jiang MM, Zhang ZB (2009) Behavioral adaptation of Pallas's squirrels to germination schedule and tannins in acorns. Behav Ecol 20:1050–1055

Xiao ZS, Jansen PA, Zhang ZB (2006a) Using seed-tagging methods for assessing post-dispersal seed fate in rodent-dispersed trees. For Ecol Manag 223:18–23

Xiao ZS, Wang YS, Harris M, Zhang ZB (2006b) Spatial and temporal variation of seed predation and removal of sympatric large-seeded species in relation to innate seed traits in a subtropical forest, Southwest China. For Ecol Manag 222:46–54

Xiao ZS, Zhang ZB, Wang YS (2005) Effects of seed size on dispersal distance in five rodent-dispersed fagaceous species. Acta Oecol 28:221–229

Yang XP, Xiao YX (2002) Animal Physiology. Higher Education Press, Beijing

Yi XF, Xiao ZS, Zhang ZB (2008) Seed dispersal of Korean pine *Pinus koraiensis* labeled by two different tags in a northern temperate forest, northeast China. Ecol Res 23:379–384

Yi XF, Zhang ZB (2008) Seed predation and dispersal of glabrous filbert (*Corylus Heterophylla*) and pilose filbert (*Corylus Mandshurica*) by small mammals in a temperate forest, northeast China. Plant Ecol 196:135–142

Zera AJ, Harshman LG (2001) The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95–126

Zhang HM, Zhang ZB (2008) Endocarp thickness affects seed removal speed by small rodents in a warm-temperate broad-leafed deciduous forest, China. Acta Oecol 34:285–293

Zhang HM, Chen Y, Zhang ZB (2008) Differences of dispersal fitness of large and small acorns of Liaodong oak (*Quercus liaotungensis*) before and after seed caching by small rodents in a warm temperate forest, China. For Ecol Manag 255:1243–1250

Zhang Z, Wang F (2000) Effect of burial on acorn survival and seedling recruitment of Liaodong oak (*Quercus liaotungensis*) under rodent predation. Acta Theriol Sin 21:35–43

Zweifel-Schielly B, Leuenberger Y, Kreuzer M, Suter W (2012) A herbivore's food landscape: seasonal dynamics and nutritional implications of diet selection by a red deer population in contrasting Alpine habitats. J Zool 286:68–80