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Behavioural responses to acorn germination by tree squirrels in an old forest where white oaks have long been extirpated

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Many plants and animals have evolved physical and/or chemical defences to protect them from a wide range of predators, and in turn these predators have also developed effective offensive strategies in morphology, physiology and behaviour to counterbalance the evasive strategies of their prey. However, little is known about how morphology, physiology and behaviour in either predator or prey respond to local extinction of their interacting partners. Interactions between scatter-hoarding squirrels and oaks provide a valuable model system to explore such questions. In an ancient forest in southwest China, where nondormant white oaks have long been extirpated, we investigated how free-ranging Asian red-cheeked squirrels, *Dremomys rufigenis*, manipulated acorn germination, even when they had no experiences of white oak acorns in their habitat. We also tested the food perishability hypothesis, which suggests that rapid germination of seeds can lead to loss of energy and nutrients during storage. Our results were mostly consistent with the food perishability hypothesis: like several other squirrel species in both Asia and North America, (1) the focal squirrels were able to distinguish subtle differences between dormant and nondormant acorns, with more nondormant acorns having their embryos removed and dormant acorns being hoarded more; and (2) embryo-removed acorns had significantly lower germination success. Our study suggests that embryo removal behaviour by scatter-hoarding squirrels may be an adaptive trait allowing squirrels to use nondormant acorns as a long-term food supply and such adaptive behaviour can remain unchanged even when white oaks are extinct in their habitats.

Many plants and animals have evolved physical and/or chemical defences to protect them from a wide range of predators, and in turn these predators have also developed effective offensive strategies in morphology, physiology and behaviour to counterbalance the evasive strategies of their prey (Abrams 2000; Karban & Agrawal 2002; Brodie & Ridenhour 2003). In many predator–prey systems, loss of one interacting partner may have substantial impacts on the abundance and distribution of the other partner (e.g. Paine 1969; Power et al. 1996). However, little is known about how morphology, physiology and behaviour in either predators or prey respond to local extinction of their interacting partners. In general, prey selection is the first step in understanding the ecological and evolutionary interplay between predator and prey. According to optimal foraging theory, the ability to select prey should be heritable in some ways for predators (Stephens & Krebs 1986). If prey selection is under genetic control for predators, we argue that such selection may remain unchanged even when related prey have been extinct for a long time in the habitat.

For hoarding animals, as both seed predators and dispersers, rapid germination (i.e. nondormancy) of seeds (as prey) is one major cause of food perishability owing to energy and nutrients being lost during storage (the food perishability hypothesis, e.g. Steele et al. 2001a, 2006; Jansen et al. 2006; Xiao et al. 2009; Cao et al. 2011). Produced by oak species (*Quercus* sensu lato, Fagaceae) across the North Hemisphere, acorns exhibit two relatively distinct germination strategies among the three well-studied oak groups: most white oaks (section *Quercus*, hereafter referred to as WO) in both Eurasia and North America produce nondormant acorns, while red oaks (section Lobatae, RO) in North America and Qinggang oaks (section *Cyclobalanopsis*, QG) in Asia often produce dormant acorns (e.g. Fox 1982; Smallwood et al. 2001; Steele et al. 2001b; Z. Xiao, unpublished data). A number of squirrel species are known to remove the embryos of nondormant WO acorns, for example in North America from the genus *Sciurus*: eastern grey squirrel, *S. carolinensis*, red-bellied squirrel, *S. aureogaster*, and fox

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squirrel, *S. niger* (Fox 1982; Steele et al. 2001a), and in China, Pallas's squirrel, *Callosciurus erythraeus*, and Pére David's rock squirrel, *Sciurotamias davidianus* (Xiao et al. 2009, 2010). Since these scatter-hoarding squirrels have evolved similar adaptive behaviour (here embryo removal) in response to acorn germination either within or across continents, we expected other species from the squirrel family (Sciuridae) should also have evolved embryo removal behaviour if they have been closely associated with nondormant WO acorns in their habitats. In general, any adaptive traits in morphology, physiology and behaviour may have been moulded by phylogenetic histories and/or strong selective forces (McKitrick 1993; Schluter 2000). Therefore, embryo removal behaviour by these scatter-hoarding squirrels is expected to be an evolutionarily conserved or convergent trait in relation to the evolution of non-dormancy in WOs over time and space.

Although other traits (e.g. seed size and tannin level) in acorns show some significant effects on squirrels' hoarding behaviour, the food perishability hypothesis based on germination schedules provides a reasonable explanation for why the squirrels named above display embryo removal behaviour to nondormant WO acorns (e.g. Hadj-Chikh et al. 1996; Smallwood et al. 2001; Steele et al. 2006; Xiao et al. 2009, 2010). There are three key predictions derived from the food perishability hypothesis: (1) nondormant acorns are more likely to be consumed in situ or have their embryo removed prior to hoarding; (2) dormant acorns are hoarded more than nondormant acorns; and (3) embryo removal should reduce acorn germination if embryo-removed acorns are used as long-term storage (Fox 1982; Hadj-Chikh et al. 1996; Smallwood et al. 2001; Steele et al. 2001a, b, 2006; Xiao et al. 2009, 2010). However, it is unknown whether embryo removal behaviour shown to nondormant acorns is a genetically based trait in these squirrels. Recently, Steele et al. (2006) provided some evidence for this hypothesis based on the eastern grey squirrel. They found that both naïve and wild-caught squirrels prefer to hoard more dormant RO acorns than WO acorns, and naïve squirrels also display embryo removal behaviour to nondormant (germinated) acorns. If squirrels' responses to acorns are heritable, as Steele et al. (2006) suggested, we also contend that our study squirrels and their descendants should display the same suite of behavioural responses (embryo removal in particular) to nondormant WO acorns even when WOs have been extinct in their habitats for a long time.

In this study, we investigated how free-ranging Asian red-cheeked squirrels, *Dremomys rufigenis*, manipulated acorns with contrasting germination strategies by presenting acorn pairs from one WO species (i.e. *Quercus variabilis*) and two QG species (*Cyclobalanopsis stewardiana* and *C. glaucoides*) in an ancient forest in southwest China, where WOs have long been extirpated. Asian red-cheeked squirrels (body mass, ca. 250–350 g) are widely distributed in the forests with or without WOs in Southeast Asia, including southern China (Wang 2003; Wilson & Reeder 2005; Z. Xiao, personal observation). We addressed the following questions based on the food perishability hypothesis. (1) Do Asian red-cheeked squirrels display embryo removal behaviour to nondormant WO acorns? (2) Do the squirrels prefer to hoard more dormant acorns than nondormant acorns (including those germinated)? (3) Is embryo removal efficiency of nondormant acorns by Asian red-cheeked squirrels similar to that of other squirrel species, when comparing germination success between embryo-removed and intact acorns? If Asian red-cheeked squirrels living in forests without WOs show similar hoarding behaviour (embryo removal of nondormant acorns in particular) as other squirrel species, this would suggest that at least some components of such hoarding behaviour may be under genetic control, because the focal squirrel species has no actual experience of hoarding nondormant acorns.

METHODS

Study Area and Study Species

We conducted field experiments during the autumn (October–December) of 2009 in the Ailaoshan National Nature Reserve (ca. 5100 km^2; 2000–2650 m; 24°32.677′N, 101°01.669′E) in Yunnan Province, southwest China. This nature reserve was established in 1981 to protect the vegetation and the biodiversity of the area. The total annual rainfall is about 193.1 cm with frequent rainfall from May to October. The average annual temperature is 11.3 °C with low temperatures of 5.4 °C in January and high temperatures of 16.4 °C in July. The main natural vegetation found in the core area of the nature reserve, Xujiaba (Fig. S1 in the Supplementary material), is old growth, montane moist evergreen broadleaved forest, which is dominated by *Lithocarpus* spp. (e.g. *L. xylocarpus* and *L. hancei*), *Castanopsis wattii*, *Hartia sinensis*, *Machilus viridis*, *Manglietia insignis* and *Schima noronhae* as canopy trees and *Camellia forrestii*, *Eriobotrya bengalensis*, *Eurya obliquifolia*, *Litsea elongata*, *Rhododendron leptothrium*, *Vaccinium duclouxii* and *Symplocos* spp. as short trees or shrubs (Wu 1983; Qiu et al. 1998). At Xujiaba, there is a small population of two Qinggang oaks, *C. stewardiana* and *C. myrsinifolia*, with very few fruiting individuals, but other Qinggang oaks, such as *C. glaucoides* and *C. glauca*, are also distributed in secondary forests nearby (Zhu & Yan 2009). WOs, such as *Q. variabilis* and *Q. franchetii*, have been extirpated for at least several hundred years at Xujiaba, although these two WOs are found in some secondary forests near the border of Xujiaba, but at least 5 km away (Qiu et al. 1998; Z. Xiao, personal observation).

At the study site, common rodent species include the Asian red-cheeked squirrel, the Chinese white-bellied rat, *Niviventer confucianus*, the Anderson's white-bellied rat, *N. andersoni*, and the South China field mouse, *Apodemus draco* (Wu et al. 1983; Z. Xiao, unpublished data). These rodents rely heavily on tree seeds especially from Fagaceae species (e.g. *Lithocarpus* spp. and *Castanopsis* spp.) and other large-seeded species as important food sources. According to a recent survey (2006–2010), Asian red-cheeked squirrels and possibly other rodent species (e.g. South China field mouse) scatter-hoard tree seeds when soil/ground surfaces are disturbed or when presented with tagged seeds on the forest floor. However, there is no information about the foraging behaviour of Asian red-cheeked squirrels, although craniodental morphology and related muscles suggest that they can consume hard seeds and tough tree bark (Koyabu et al. 2009). At Xujiaba, Asian red-cheeked squirrels often feed and hoard dormant nuts from *Lithocarpus* spp. and *Castanopsis* spp. However, it is unlikely that they have had experience of feeding on and hoarding nondormant WO acorns because WOs (e.g. *Q. variabilis*) have been extirpated for hundreds of years. In the autumn of 2009, we found that this squirrel species was highly sensitive to nondormant acorns from one WO species *Q. variabilis* and frequently removed their embryos, and it also occasionally removed the embryos of dormant acorns from Qinggang oaks such as *C. stewardiana* and *C. glaucoides* (Fig. 1; see below).

Behavioural Experiments

In this study, acorns from three oak species were used in behavioural experiments based on their availability: one WO species *Q. variabilis* (QV) and two QG species (*C. stewardiana*, CS and *C. glaucoides*, CG). QV acorns, like most WOs, often germinate soon after seed fall, while acorns from CG and CS, like red oaks in North America, often exhibit dormancy until the following spring. The three oak species used in this study produce high-tannin but low-fat acorns (Table 1). However, two of the three acorn species,

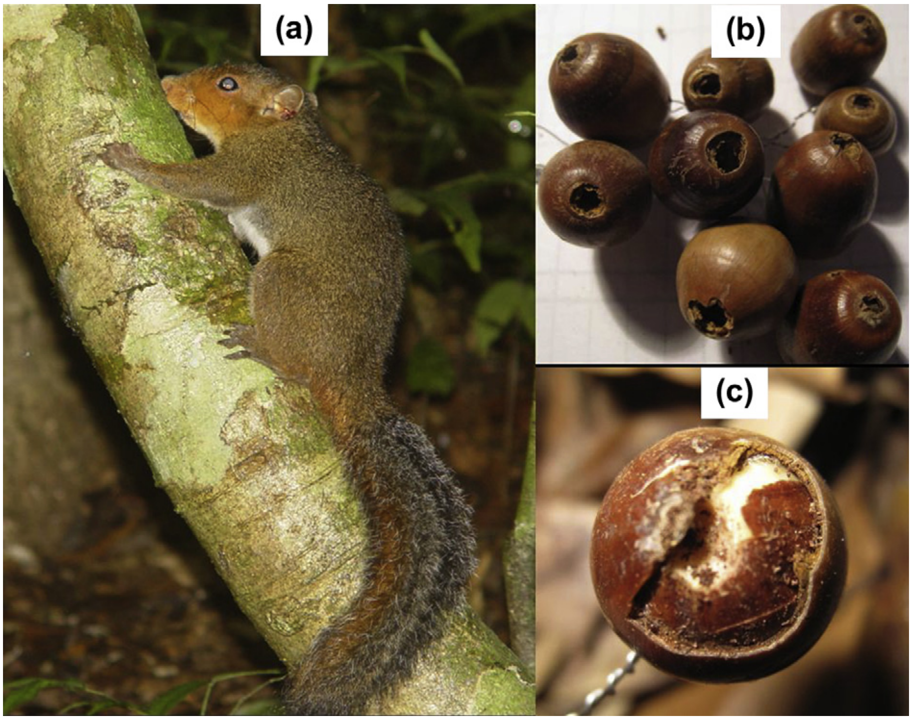

Figure 1. (a) Asian red-cheeked squirrel. Photo: Lin Cao. (b, c) Embryo-removed acorns from (b) white oak and (c) qinggang oak.

QV and CS, were larger than CG acorns (Table 1). We collected CS acorns at the study site (i.e. Xujiaba) and CG and QV acorns from the secondary forests approximately 30 km away, and stored them in relatively dry sand (storing acorns in sand is an easy way to keep seeds healthy in natural conditions, when other means such as refrigeration are not available). In this study, only sound acorns were used for behavioural experiments and any acorns infested with weevils or microbes were discarded.

We performed three types of behavioural experiments to see how Asian red-cheeked squirrels handled acorns with contrasting germination strategies: (1) no-choice trial with only nongerminated or germinated QV acorns; (2) paired-choice trial with equal numbers of nongerminated and germinated QV acorns; and (3) paired-choice trial with equal numbers of nongerminated CG or CS acorns and nongerminated or germinated QV acorns. Here germinated acorns (g) had radicles protruding from the pericarp (QV acorns only), but nongerminated acorns (ng) showed no visible signs of germination (QV acorns) or remained dormant (CQ and CS acorns) during the experimental period. In addition, nongerminated QV acorns are somewhat different from dormant QG/RO acorns because squirrels are able to detect the subtle differences between nongerminated WO acorns and dormant QG/RO acorns in both China and North America (see also Steele et al. 2006; Xiao et al. 2010; Z. Xiao, unpublished data). For no-choice trials, we presented free-ranging squirrels with 50 nongerminated QV acorns or 50 germinated QV acorns. For paired-choice trials, we presented the animals with equal numbers (i.e. 25:25) of both nongerminated and germinated acorns from either the same species (i.e. QVng versus QVg) or different species (i.e. CGng versus QVng; CGng versus QVg; CSng versus QVng; and CSng versus QVg). We performed three replicates for each of the three trials. For each replicate ($N = 21$ in total), we placed 50 tagged acorns randomly distributed in a circle (diameter ca. 0.5 m) on the ground. All replicates were performed randomly at one of 10 different plots (separated by 100–3000 m), and each one was conducted at a different location to avoid any biases that would result from the squirrels' experience. See similar procedures in Xiao et al. (2010).

Individual acorns were labelled with a numbered plastic tag attached by a thin stainless steel wire 10 cm long, similar to the procedures reported by Xiao et al. (2006; see also Xiao et al. 2008, 2009, 2010). Because squirrels are diurnal, we began between 0750 and 0850 hours and monitored tagged acorns until 1530 hours. At each feeding station, acorn fate was determined from the centre of the station to within a radius of approximately 30 m. Acorns at each station were categorized as (1) remaining or (2) not removed but eaten, and those removed from the station were categorized as (3) hoarded, (4) eaten (tags and seed fragments found) or (5) missing (not retrieved). Hoarded acorns were excavated to identify whether embryos were removed by the squirrel or not. Distances to the source were measured for the hoarded and the eaten acorns. To identify which animal species were responsible for the hoarding and embryo removal of the tagged acorns, we used a digital Video Camera (Sony DCR-SR85E) for some replicates ($N = 4$, ca. 10 h in total). Video camera documentation, live trapping and daily experiments confirmed that the Asian red-cheeked squirrel was the only animal species harvesting the tagged acorns at the study site.

The study was approved by the Animal Research Ethics Board of the Institute of Zoology, Chinese Academy of Sciences.

Table 1
Acorn traits of white oak *Quercus variabilis* and qinggang oaks *Cyclobalanopsis glaucoides* and *C. stewardiana* used in this study

Oak species	Germination schedule	Fat (% by dry weight)	Tannin (% by dry weight)	Seed size (mean±SE g)
*Quercus variabilis**	Nondormant	3.94	11.68	2.36±0.02
Cyclobalanopsis stewardiana†	Dormant	0.99	15.55	2.29±0.08
Cyclobalanopsis glaucoides†	Dormant	2.41	7.86	1.70±0.02

* Xiao et al. (2003).
† This study.

Germination Experiments

We determined to what extent embryo removal by the Asian red-cheeked squirrel deterred acorn germination compared with two other squirrel species, the Pallas's squirrel in the Qingchengshan area and the Pére David's rock squirrel in the Qinglin area. Using the seed-tagging method (see above), we collected *Q. variabilis* acorns from which the embryos had been excised by each of the three squirrel species: 90 acorns from the Asian red-cheeked squirrel, 35 acorns from the Pallas's squirrel and 50 acorns from the Pére David's rock squirrel. On 20 December 2009, all these embryo-removed acorns and another 50 intact acorns (treated in the same way as the tagged acorns) were used as a control and sown under the surface soil at a depth of 2–4 cm, like most squirrel-made caches, in three rodent-proof wire cages (area 1 m^2; mesh size 1×1 cm). Three months after sowing, we carefully excavated all the acorns, and recorded whether they were germinated or not. Using intact acorns as a control, we identified normally germinated acorns by the presence of one or two strong taproots with some small fibres, while most of the embryo-removed acorns were not germinated or produced a few thin roots or fibres (unlike taproots from intact acorns) but they were still edible for hoarding animals.

Data Analysis

In this study, each germination type of QV acorns was tested in up to 12 replicate trials and dormant CS or CG acorns were also tested in six replicates, although each trial had only three replicates. However, Asian red-cheeked squirrels showed similar patterns in response to QV acorns (either germinated or non-germinated) and dormant CS or CG acorns when these acorns were presented, either independently (i.e. no-choice trials) or paired with other acorns (i.e. paired-choice trials; Fig. 2).

In this study, we examined three variables to measure squirrels' hoarding behaviour. For hoarding probability (i.e. the proportion of acorns hoarded) and embryo removal probability (i.e. the proportion of hoarded seeds with embryo removal), generalized linear mixed models were used with a logit link function, with acorn species or germination phenotype as a categorical factor and replicate as a random factor for each choice trial. However, we had

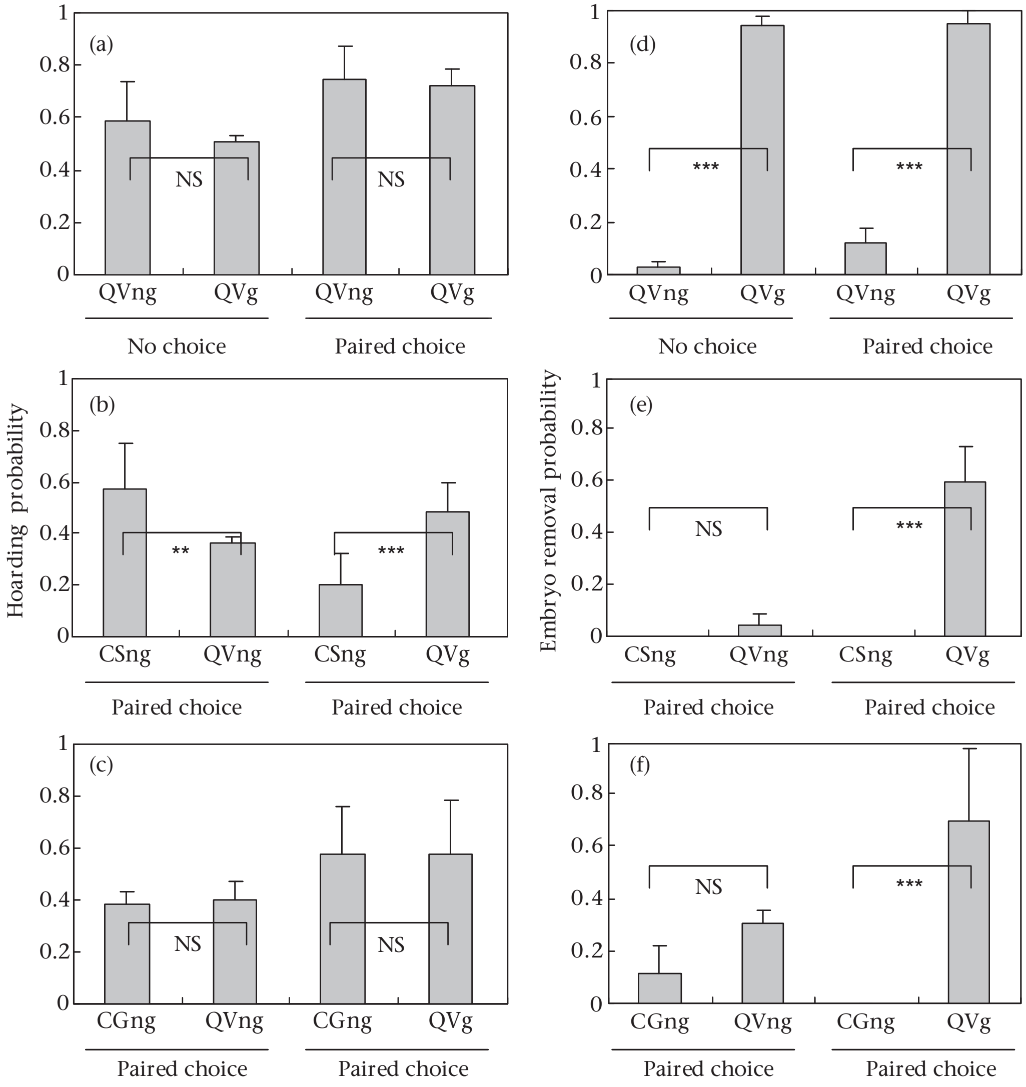

Figure 2. Behavioural responses (mean + 1 SE) to dormant (nongerminated, ng) and nondormant (germinated, g) acorns by Asian red-cheeked squirrels. (a, b, c) Hoarding probability (i.e. the proportion of acorns that were hoarded). (d, e, f) Embryo removal probability (i.e. the proportion of hoarded seeds with embryo removal). (a) No-choice trials for either nongerminated or germinated acorns from white oak, *Quercus variabilis* (QV) and paired-choice trials for nongerminated and germinated QV acorns. (b–f) Paired-choice trials for nongerminated Qinggang acorns from either *Cyclobalanopsis glaucoides* (CG) or *Cyclobalanopsis stewardiana* (CS) and nongerminated or germinated QV acorns. $^{**}P < 0.01$; $^{***}P < 0.001$.

to pool the data according to oak species for three paired-choice trials (i.e. CGng versus QVg; CSng versus QVng; and CSng versus QVg) because there were no dormant acorns from CG or CS with their embryos removed. Thus, Fisher's exact tests were used to test the difference in embryo removal probability between acorn pairs for each of the three paired-choice trials. We used Student's *t* test to see the differences in dispersal distance (log-transformed to meet the normality for analysis) of hoarded acorns for each paired trial.

Pearson chi-square tests were also used to test the difference in germination success between the four treatments, that is, embryo-removed acorns by each of the three squirrel species and intact acorns (control), and between any two of the four treatments.

Generalized linear mixed models were carried out in the R program (version 2.8.1, R Development Core Team 2008), and other statistical analyses were done using SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

Behavioural Experiments

Squirrels harvested nearly all of the tagged acorns and then hoarded most of them individually in shallow soil or leaf litter. For no-choice and paired-choice trials, a similar proportion of QV acorns were hoarded for each germination type (i.e. nongerminated or germinated; no-choice trial: $z = 1.430$, $P = 0.153$; paired-choice trial: $z = 0.383$, $P = 0.702$; Fig. 2a). However, more germinated acorns had their embryos removed by squirrels, compared to nongerminated ones (no-choice trial: 94.70% versus 3.13%; $z = 7.293$, $P < 0.0001$; paired-choice trial: 95.23% versus 12.22%; $z = 6.693$, $P < 0.0001$; Fig. 2d). For the no-choice trial, germinated QV acorns were dispersed further than those nongerminated ($t_{162} = 4.612$, $P < 0.001$), but in the paired-choice trial, dispersal distances were similar ($t_{108} = 0.006$, $P = 0.995$; Fig. 3a).

When CS and QV acorns were presented together, more CS acorns were hoarded than nongerminated QV acorns (63.73% versus 36.50%; $z = 2.684$, $P = 0.007$), but germinated QV acorns were hoarded more than CS acorns (48.00% versus 20.00%; $z = 3.545$, $P = 0.0004$; Fig. 2b). However, the results from the CG–QV pairs were somewhat different from the CS–QV pairs: the animals hoarded a similar proportion of acorns for each species regardless of the germination types of QV acorns (CGng versus QVng: $z = 0.167$, $P = 0.867$; CGng versus QVg: $z = 0.0001$, $P = 1.000$; Fig. 2c). In addition, germinated QV acorns had a higher embryo removal probability than those from either CS (QVng versus CSng: 3.70% versus 0%; $P = 0.386$; QVg versus CSng: 75.44% versus 0%; $P < 0.0001$; Fig. 2e) or CG (QVng versus CGng: 30.55% versus 11.11%; $z = 1.802$, $P = 0.072$; QVg versus CGng: 69.58% versus 0%, $P < 0.0001$; Fig. 2f). Moreover, germinated QV acorns also had a significantly higher embryo removal probability than nongerminated QV, when presented with either CS ($z = 3.414$, $P = 0.0006$; Fig. 2e) or CG ($z = 2.892$, $P = 0.004$; Fig. 2f).

In CS–QV paired trials, dispersal distances were significantly different between CS acorns and nongerminated QV acorns ($t_{68} = 2.823$, $P = 0.006$), but similar between CS acorns and germinated QV acorns ($t_{49} = 1.654$, $P = 0.104$; Fig. 3b). In CG–QV paired trials, dispersal distances of CG acorns were similar to those of both nongerminated QV acorns ($t_{57} = 1.759$, $P = 0.084$) and germinated QV acorns ($t_{84} = 1.078$, $P = 0.284$; Fig. 3c).

Germination Experiments

Three months after they were sown, no more than 20% of *Q. variabilis* acorns successfully germinated for those with the embryo removed by any of the three squirrel species, and this was significantly lower than for intact acorns (88%; $\chi^2_3 = 107.474$, $P < 0.001$; Fig. 4). Acorns with the embryo removed by Asian red-cheeked squirrels and Pallas's squirrels had a similar probability of germinating normally ($P = 1.00$), but those handled by Pére David's rock squirrels had a much lower germination probability than those handled by either Asian red-cheeked squirrels ($\chi^2_3 = 8.879$, $P = 0.003$) or Pallas's squirrels ($\chi^2_3 = 7.804$, $P = 0.005$) or intact acorns ($\chi^2_3 = 74.707$, $P < 0.001$).

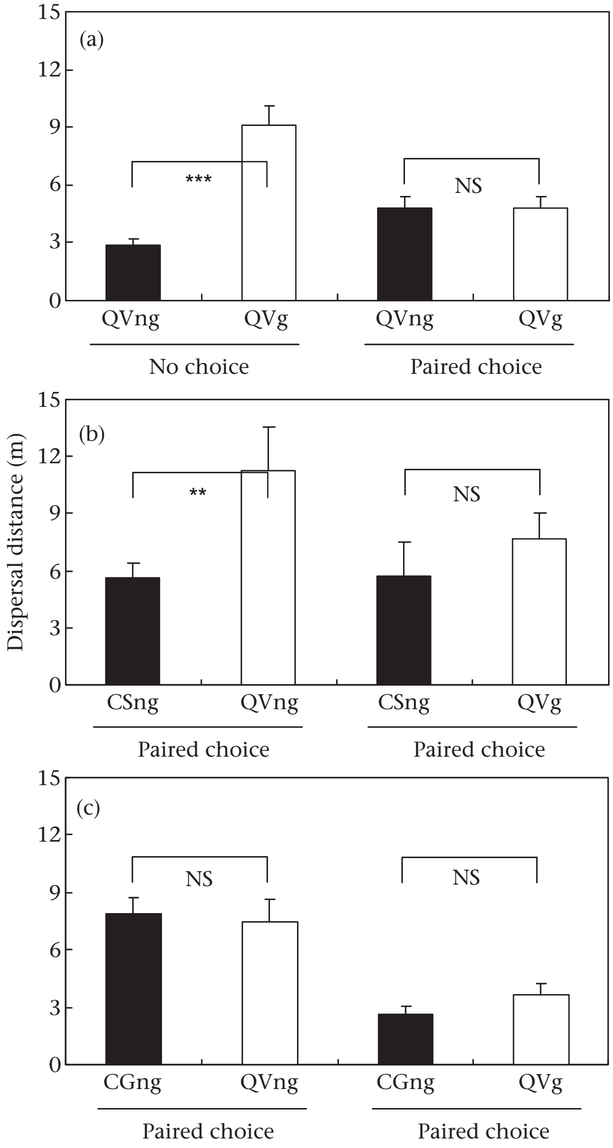

Figure 3. Dispersal distances (mean + 1 SE, m) of nongerminated (ng) and germinated (g) acorns hoarded by squirrels when both acorn types were paired. (a) No-choice trials for either nongerminated or germinated acorns from white oak, *Quercus variabilis* (QV) and paired-choice trials for nongerminated and germinated QV acorns. (b, c) Paired-choice trials for nongerminated Qinggang acorns from either (b) *Cyclobalanopsis stewardiana* (CS) or (c) *Cyclobalanopsis glaucoides* (CG) and nongerminated or germinated QV acorns. $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

By presenting acorn pairs with contrasting germination strategies or phenotypes in the Ailaoshan forest where WOs have long been extirpated, we have provided clear evidence that, like other squirrel species reported in both Asia and North America, Asian

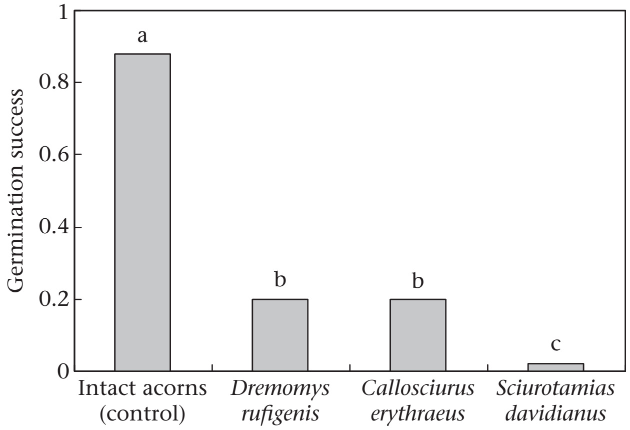

Figure 4. A comparison of germination success of *Quercus variabilis* acorns whose embryos are removed by three squirrel species in China: the Asian red-cheeked squirrel in the Ailaoshan area, Pallas's squirrel in the Qingchengshan area and the Pére David's rock squirrel in the Qinglin area. Acorn germination was detected 3 months after being sown. 'Control' refers to acorns with intact embryos. Bars with different letters indicate a significant difference ($P < 0.05$) between them.

red-cheeked squirrels showed a similar suite of behavioural responses (including embryo removal) to acorn germination although they had no experience of hoarding nondormant acorns. Our results were mostly consistent with the food perishability hypothesis. First, Asian red-cheeked squirrels were able to distinguish subtle differences between dormant and nondormant acorns. Fewer nongerminated QV acorns (0–30%) had their embryos removed than germinated ones (50–100%), when they were either presented independently (i.e. no-choice trials) or paired with each other or with dormant QG acorns (i.e. paired-choice trials; Fig. 2). Second, the study squirrels not only hoarded more dormant acorns, but also hoarded a large proportion of nondormant acorns (including those germinated) but with their embryos removed in most cases. Third, embryo removal by Asian red-cheeked squirrels significantly reduced acorn germination, and the embryo removal efficiency was similar to that of the Pallas's squirrels, but relatively less than that of the Pére David's rock squirrels. In North America, embryo removal by eastern grey squirrels was also found to reduce germination success significantly (<18%) for those acorns with the embryo removed (Wood 1938; Fox 1982; McEuen & Steele 2005). This indicates that embryo-removed acorns remained longer as stored food for these squirrel species. In addition, we also found that embryo removal of nondormant QV acorns was not affected by the presence of dormant QG acorns (e.g. *C. stewardiana* or *C. glaucoides*). Therefore, for scatter-hoarding squirrels, embryo removal may be the prerequisite for nondormant acorns used as a long-term food supply.

Our results also provide some important evidence for the idea that at least some components of acorn-hoarding behaviour (embryo removal of nondormant acorns in particular) may have a genetic basis among these squirrels even when they have had no experience in their own habitats (see also Steele et al. 2006). There are several reasons to confirm this hypothesis with Asian red-cheeked squirrels. First, it is highly unlikely that the focal population had the opportunity to learn how to manipulate acorns with different germination strategies since WOs have been absent for hundreds of years. On the one hand, dormant nuts, produced by dominant trees such as *Lithocarpus* spp. and *Castanopsis* spp. (Jin 1983; Qiu et al. 1998), are the main food source for the focal squirrels at the study site (Z. Xiao, personal observation). On the other hand, the focal squirrels often consumed nearly all the acorn crops from QGs (i.e. *C. stewardiana* and *C. myrsinifolia*) when they were on the tree before maturity in both lean and rich years, because the two QGs had very few fruiting individuals at the study site and their acorn masting occurred only in 2009 during the past 5 years (i.e. 2006–2010; Z. Xiao, personal observation).

Second, it is also unlikely that during the experimental period, the focal population emigrated from the nearby secondary forests with WOs where the squirrel population may have some experience of nondormant WO acorns (e.g. QV). Our behavioural experiments were conducted in the core area of Xujiaba. Our results suggest that the study squirrels may lack feeding and hoarding experience of nondormant acorns: (1) embryo removal by the study squirrels was primarily performed on nondormant QV acorns (especially those that germinated); (2) the embryo removal efficiency in relation to germination success was relatively low, at least compared with that of the Pére David's rock squirrels; and (3) the study squirrels did not show a strong pattern of hoarding more dormant acorns than nondormant acorns (Fig. 2). According to our previous and current (2011) surveys of local people and secondary forests around Xujiaba (Z. Xiao & Z. Zhang, unpublished data), only a few fruiting individuals of *Q. variabilis* occur in a nearby village (ca. 5 km away from Xujiaba), because, for hundreds of years, local people have converted natural forests into farmland for agricultural crops and plantations of economic trees (e.g. walnuts). This suggests that the whole population of the study squirrels at Xujiaba and secondary forests nearby may have a very limited experience of feeding on and hoarding nondormant WO acorns. Although the nearest distance from the border of Xujiaba to the site with WOs was relatively short (ca. 5 km), the migration route may have been more than 10 km, because there are several large ridges and valleys between the site with WOs and the study site (Wu 1983; Fig. S1 in the Supplementary material). Since Asian red-cheeked squirrels showed embryo removal of nondormant WO acorns even when they had no experience of these acorns in their habitat, our results suggest that the ancestors of the study squirrels should have had a long history of close interactions with WOs.

Third, we have no data to confirm that Asian red-cheeked squirrels may learn from the Pallas's squirrels. The distributions of the two squirrel species frequently overlap in subtropical and tropical forests in China (see Wang 2003; Wilson & Reeder 2005; Z. Xiao, personal observation) and the Pallas's squirrels display embryo removal behaviour to nondormant acorns (Xiao et al. 2009; Z. Xiao, unpublished data). According to our 6-year survey (2006–2011) based on video camera documentation, live trapping and daily experiments, we did not find any individual Pallas's squirrels at the study site. Since these two squirrel species in China have been found to show the same acorn-hoarding behaviour in relation to acorn germination, we contend that the common ancestors of these two squirrel species may have evolved such adaptive behaviour in response to the evolution of nondormant acorns. Therefore, if this behaviour was derived from a common ancestor, it is unlikely that these two squirrel species learned such acorn-hoarding behaviour from each other.

Fourth, embryo removal of nondormant acorns by scatter-hoarding squirrels may be an evolutionarily conserved trait in the squirrel family Sciuridae because there are a number of squirrel species (presently including the four genera *Sciurus*, *Callosciurus*, *Sciurotamias* and *Dremomys*) that are found to display such behaviour either within or across continents. If this behaviour is not under genetic control and is learned from experienced individuals or species, it is hard to image why in both China and North America, so many squirrel species show embryo removal behaviour in relation to nondormant WO acorns, but many other rodent species such as rats, mice and chipmunks do not, even when these rodent species co-occur with the former squirrel species in the same habitats. However, learning may also be important for young squirrels to improve their embryo removal ability and other hoarding behaviour when they use nondormant acorns as food storage (see Fox 1982; Steele et al. 2006). This remains to be studied in the future.

In conclusion, our study with Asian red-cheeked squirrels provides supportive evidence that embryo removal behaviour may be an adaptive trait allowing scatter-hoarding squirrels to use nondormant WO acorns as a long-term food supply and such adaptive behaviour can remain unchanged even when WOs are extinct in the habitat. Studies from both Asia and North America hold that the ability to remove the embryos of nondormant WO acorns selectively is a counter-adaptation for scatter-hoarding squirrels (Fox 1982; Steele et al. 2001a, b, 2006; Xiao et al. 2009, 2010; this study). These studies also underscore that the evolution of germination schedules in acorns (and other seeds) may act as the primary signal to induce the evolution of specific foraging behaviour such as embryo removal among seed predators/dispersers (see also Jansen et al. 2006; Cao et al. 2011). Moreover, these studies indicate that embryo removal behaviour may be restricted to the squirrel family. Therefore, it is expected that such adaptive behaviour may have evolved early in the squirrel family and may be evolutionarily conserved across many of the genera in this family.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.anbehav.2012.01.013.

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