

Dual ecological functions of scatter-hoarding rodents: pollinators and seed dispersers of *Mucuna sempervirens* (Fabaceae)

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Abstract

Double mutualism, that is, pollination and seed dispersal of the same plant species mediated by the same animal partners, is important but remains elusive in nature. Recently, rodent species were found as key pollinators (i.e. explosive openers) for some *Mucuna* species in (sub)tropical Asia, but no evidence has shown whether and how these rodents could also act as legitimate seed dispersers via scatter-hoarding for those producing large seeds. Here, my aim was to test the hypothesis that scatter-hoarding rodents could act as double mutualists for both pollination and seed dispersal of the same *Mucuna* species, that is, *Mucuna sempervirens* (Fabaceae). Based on camera-trapping survey at 2 locations with or without squirrel presence in the Dujiangyan subtropical forests, Southwest China, 7 mammals and birds were identified as explosive openers for *M. sempervirens* flowers, but *Leopoldamys edwardsi* (rats) and *Paguma larvata* (civets) were the main pollinators at the squirrel-absent site, while *Callosciurus erythraeus* (squirrels) were the main pollinators at the squirrel-present site. By tracking the fate of individually-tagged seeds over 5 years at each site, I provide the first evidence for seed-eating rodents as legitimate seed dispersers via scatter-hoarding of seeds in this world-wide plant genus, although dispersal services were slightly reduced at squirrel-absent site. More importantly, the dual roles of scatter-hoarding rodents as key pollinators and seed dispersers for the same *Mucuna* species have shown a clear relationship of double mutualism, and their key services may be essential for population conservation of these *Mucuna* species in human-disturbed landscapes.

Key words: double mutualism, *Mucuna* (Fabaceae), pollination, scatter-hoarding rodents, seed dispersal

INTRODUCTION

Pollination and seed dispersal by animal vectors are key ecosystem functions for the maintenance of plant re-

production success, and population and community dynamics (Herrera & Pellmyr 2002). Different animal taxa can act as either legitimate pollinators or seed dispersers for many flowering plants across the world (Herrera & Pellmyr 2002; Ollerton *et al.* 2011). In some cases, dual ecological functions of a single plant species can be served by the same animal species (e.g. Kelly *et al.* 2004; Hansen & Müller 2009; Nakamoto *et al.* 2009; Gomes *et al.* 2014; Olesen *et al.* 2018; Fuster & Traveset 2019, 2020). Such phenomena are recognized as “double mutualism” (Hansen & Müller 2009). According to

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a global review (Fuster *et al.* 2019), double mutualism phenomena may be more prevalent in island ecosystems with limited food resources and mutualist partners, and also in tropical regions with higher generalization levels. In many cases, birds may often act as double mutualists for those plants producing actinomorphic flowers and fleshy fruits. Due to limited cases and geographic coverage, however, the role of double mutualism phenomena in plant population and community dynamics still remain elusive in nature, for example, why double mutualism is rarely found in dry-fruited plants and is less common in mainland ecosystems, and how effective double mutualists are in both processes (Fuster *et al.* 2019).

The world-wide genus *Mucuna* (Fabaceae), including over 100 vine species, is primarily distributed in (sub)tropical regions (Lewis *et al.* 2005). The key pollination feature of most *Mucuna* species is highly dependent on specific animal vectors to initiate explosive opening to their flowers, where animal vectors must open each flower to squeeze the wing petals and the banner petal using considerable force (van der Pijl 1941; Agostini *et al.* 2006; Toyama *et al.* 2012; Kobayashi *et al.* 2018c). Otherwise, these *Mucuna* species cannot produce viable seeds if their flowers are not explosively opened (Kobayashi 2018). Based on literature reports, primates, bats, martens, civets, and rodents are demonstrated as key pollinators (i.e. explosive openers) for some *Mucuna* species (belonging to *Mucuna* subg. *Macrocarpa*, see Moura *et al.* 2016a) in (sub)tropical Asia (e.g. Hopkins & Hopkins 1993; Kobayashi *et al.* 2019a), while bats and birds are key pollinators in other parts of tropical regions (see recent review by Kobayashi 2018). Though *Mucuna* pollination has been studied on different continents, whether and how animal dispersal agents and seed predators influence population dynamics of this world-wide plant genus has rarely been explored (but see Kuprewicz & Garcia-Robledo 2010; Kuprewicz 2013). When considering the double mutualism, one key question is whether and how these mammal and bird pollinators can also act as legitimate seed dispersers for the same *Mucuna* species.

Many *Mucuna* species produce woody pods and each pod contains one to several large nutritious seeds with hard, woody coats (Sa & Wilmot-Deer 2010; Yang & Zhou 1992). To the best of my knowledge, however, no clear evidence about seed dispersal by animal vectors is available for this world-wide genus *Mucuna*. Kuprewicz and Garcia-Robledo (2010) provide evidence for

bat-pollinated *M. holtonii*, the seeds of which are mainly consumed by the collared peccary (*Pecari tajacu*) in a Costa Rican rain forest, but not consumed by scatter-hoarding Central American agoutis (*Dasyprocta punctata*). Kobayashi *et al.* (2020) showed that sika deer (*Cervus nippon*) can feed on the flowers and young pods of *M. macrocarpa* near the ground level. They further implied 3 candidate methods for *Mucuna* seed dispersal: (1) gravitational dispersal after release from the split pods, (2) water dispersal along the riverside or other waterbody (ocean), and (3) mammalian dispersal based on gnawed seed coats, signs of animal activity. Among these, gravitational and mammalian dispersal may be important for local population dynamics, while water dispersal may be important for long-distance dispersal and even continental colonization (Zies 1999; Moura *et al.* 2016b). Recently, several rodent species have been identified as key pollinators for at least 4 *Mucuna* species (*M. birdwoodiana*, *M. macrocarpa*, *M. sempervirens*, and *M. championii*) in tropical and subtropical Asia (Table S3, Supporting Information; Chen *et al.* 2012; Kobayashi 2018; Kobayashi *et al.* 2019b, 2020, 2021). However, *Mucuna* seed dispersal or consumption by scatter-hoarding rodents has not yet been documented. If these rodents (notably squirrels) are confirmed as legitimate seed dispersers via scatter-hoarding for the same *Mucuna* species producing large seeds, we would expect a double mutualism phenomenon in these rodent-pollinated *Mucuna* species.

As both animal pollinators and seed dispersers are globally declining (McConkey *et al.* 2012; Regan *et al.* 2015; Corlett 2017), defaunation and other drivers of global changes are expected to increase the risk of reducing functions within these double mutualism systems (Fuster *et al.* 2019). Consequently, both pollination and dispersal limitation may be more common than expected when key double mutualists are lost in some island ecosystems or human-disturbed landscapes. Previous observations indicate that some *Mucuna* species do not produce seeds due to pollination failure in areas where key pollinators (e.g. squirrels) are lost (Chen *et al.* 2012; Kobayashi *et al.* 2015b, 2018a; personal observation by the author). If seed production is limited especially in human-disturbed landscapes, this could further cause dispersal failure in these *Mucuna* species. Therefore, it is expected that rats and mice may act as important candidate pollinators and seed dispersers of *Mucuna* populations if pollinating squirrels are extirpated in human-disturbed regions.

In this study, my aim is to test the hypothesis that scatter-hoarding rodents play a major role as double mutualists for both pollination and seed dispersal of the same *Mucuna* species on the Eurasian mainland where no double mutualism cases have been reported (see recent review in Fuster *et al.* 2019). Field surveys at 2 locations in Southwest China were used to document vertebrate pollination and seed dispersal of *M. sempervirens*. Specifically, I aimed: (1) to identify which rodent species and other mammal/bird species act as legitimate pollinators (i.e. explosive openers); (2) to measure whether and how pollinating rodents could act as legitimate seed dispersers; and (3) to assess whether and how other rodent species could fill (sensu Fedriani *et al.* 2020) the role of squirrels as both key pollinators and seed dispersers of *M. sempervirens* in habitats where squirrels are absent. Based on this study and literature reports about *Mucuna* species, I also discussed how to conserve the double mutualisms between *Mucuna* species and their animal partners in human-disturbed landscapes.

MATERIALS AND METHODS

Studies site and species

Field experiments were carried out at 2 locations with or without squirrel presence (approx. 12 km distant from each other) in Dujiangyan City of Sichuan Province, Southwest China: Qingchengshan site (area: 20 000 ha, 900–1200 m elevation, 31°03'N, 103°43'E), where native *Callosciurus erythraeus* and *Dremomys pernyi* were present, and Banruosi site (area: 300 ha, 700–1000 m, 31°04'N, 103°43'E), where squirrel species were not present, but with other rats and mice similar to those at the Qingchengshan site (Xiao & Zhang 2004, 2016; Xiao *et al.* 2009). Qingchengshan site is a national forest park, while Banruosi site is a human-disturbed area located with a mosaic of small forest stands fragmented by agricultural development, highways and villages. Climatically, Dujiangyan region lies in the middle subtropical zone, with a mean annual temperature of 15.2°C, and an annual precipitation of 1200–1800 mm. The weather is often cloudy and foggy, with only 800–1000 mean annual sunny hours and a mean annual relative humidity $\geq 80\%$. Vegetation at both locations is subtropical evergreen broad-leaf forest, and multiple species from the Fagaceae family are most common, but the wild populations of *M. sempervirens* are very small at both sites with very few individuals detected, partly because local people frequently use its woody vine as Chinese medicine.

Mucuna sempervirens, an evergreen woody vine, is widely distributed in South China, Japan, and India (Sa & Wilmot-Deaer 2010; Dong 2012). *M. sempervirens* produces large inflorescences that hang directly from old stems (named as cauliflory) at the ground level to the canopy (up to 25 m in height). Its dark purple, papilionaceous flowers (zygomorphic) are approximately 4.5 cm long, and each inflorescence has 2–21 flowers. At the studied sites, flowering period of *M. sempervirens* lasts for over 1 month (from late March to early May), and flowering phenology of this species varies among different years and sites, but with 4 main stages, that is, buds (less than 10% of inflorescences with flowers open), starting bloom (10–50% of inflorescences with flowers open), flowering open peak (50–100% of inflorescences with flowers open), and end of flowering period (over 50% of inflorescences withered and dropped with young pods emerged) (Fig. S1, Supporting Information). Like many other *Mucuna* species, explosive opening by specific animal vectors (e.g. native squirrels *D. pernyi* and *C. erythraeus*, Chen *et al.* 2012) is the key step for pollination success and pod sets in *M. sempervirens*. Chen *et al.* (2012) also showed that some *M. sempervirens* individuals did not produce any pods at one location where squirrel species were extirpated in Kunming, Southwest China. Thus, squirrels and, maybe, other mammals would act as obligatory pollinators for explosive opening and pollination success in *M. sempervirens*. However, legitimate pollinators may be replaced among different *M. sempervirens* populations (like other species of *Mucuna* subg. *Macrocarpa* populations in Asia, Kobayashi *et al.* 2020).

Similar to some *Mucuna* species, *M. sempervirens* produce long pods with 2–15 seeds per pod, and its seeds are relatively large (mean mass, 4.4 g; range, 0.61–8.26 g) with hard, woody seed coats. *M. sempervirens* seeds contain 7.38% fat content but also have 6.5–7.1% L-Dopa (Zhou *et al.* 1988; Yang & Zhou 1992). Based on our long-term studies during the past 20 years at the studied sites, several rodent species such as *C. erythraeus*, *L. edwardsi*, *Apodemus* spp., and *Niviventer* spp. have been confirmed as important seed dispersers via scatter-hoarding for many large-seeded trees such as Fagaceae species, *Camellia* spp., and even some fleshy-fruited species (Xiao *et al.* 2003, 2009; Chang & Zhang 2011). In addition, occasional observation also showed that the squirrel *C. erythraeus* can use *M. sempervirens* seeds and flowers (nectar) as food resources at the Qingchengshan site (personal observation by the author), and thus scatter-hoarding rodents may also be expected as potential seed dispersers for *M. sempervirens* at both locations. Furthermore, our field survey also found that over 50 bird species

Table 1 Foraging behaviors for mammal/bird species visiting flowers and seeds (pods) of *Mucuna sempervirens* in the Dujiangyan forests (Qingchengshan and Banruosi), Southwest China

Mammal/bird species	Activity	Flower handling (events based on camera traps) [†]			Seed handling [#]	
		Explosive opening	Nectar robbing	Bud feeding	Seed predation	Scatter-hoarding
<i>Paguma larvata</i> (Carnivora)	Nocturnal	11	0	0	No	No
<i>Apodemus</i> spp. (Rodentia)	Nocturnal	2	0	0	Yes	Yes
<i>Leopoldamys edwardsi</i> (Rodentia)	Nocturnal	8	0	26	Yes	Yes
<i>Niviventer</i> spp. (Rodentia)	Nocturnal	5	0	0	Yes	Yes
<i>Callosciurus erythraeus</i> (Rodentia) [‡]	Diurnal	56	0	3	Yes	Yes
<i>Dremomys pernyi</i> (Rodentia) [‡]	Diurnal	2	0	0	Yes	Yes
<i>Pomatorhinus ruficollis</i> (Passeriformes)	Diurnal	2	5	0	No	No
Total events		86	5	29	-	-

[‡]The 2 squirrel species only occurred at the Qingchengshan site, and all other 4 mammal or bird species were present at both Qingchengshan and Banruosi sites (Xiao *et al.* 2014a; this study; unpublished data). [†]Flower handling behaviors were recorded and identified only using camera traps (this study). [#]Seed handling behaviors for rodent species from both field and experimental evidence have been identified with the tag-marked seeds using either camera traps or semi-natural enclosures (Xiao *et al.* 2003, 2008, 2009; Chang & Zhang 2011; Gu *et al.* 2017; this study).

and some mammal/rodent species (notably *P. larvata* and rodents) are also important frugivorous seed dispersers at the studied sites (Jiang *et al.* 2010; Lai *et al.* 2014; Li *et al.* 2020).

Identifying flower visitors and their behaviors

Field surveys were conducted at the 2 studied locations with small, wild, self-seeded *M. sempervirens* populations, where several individuals were observed producing pods during the study years. Video camera traps (Ltl-5210A and Ltl-6210MC; Shenzhen Ltl Acorn Electronics Co., Ltd.) were used to record floral visitors and their behaviors with 10 s video tips with no interruption between tips, and sensitivity set to normal when flowers or buds were available until all flowers on the inflorescences had withered and dropped. At the Banruosi site, I monitored up to 72 inflorescences from 2 individuals with 14 camera traps (total trap effort: 293 d) for over 2 years (2015–2016), while at the Qingchengshan site, I monitored 11 inflorescences from 1 individual with 4 camera traps (total trap effort: 101 d) in 2016 due to logistic dif-

ficulty for camera setting on top canopy (Table S1, Supporting Information).

All camera-trap data were deposited into the database CameraData (<http://cameradata.ioz.ac.cn>) (Xiao *et al.* 2014b) for species and behavioral identification. I obtained up to 120 video groups (independent events) with vertebrate flower visitors, in which multiple video-tips with animals less than 60 s apart were grouped into a single independent event for the same species. For each independent event, I recorded and identified the visiting vertebrate species and their foraging behaviors. In this study, flower-visiting behaviors were recognized into 5 key categories after Kobayashi *et al.* 2015a, 2019b) (Table 1):

(1) Explosive opening/pollination (successful opening with no damage to the flower), which was mostly performed by *C. erythraeus* (squirrel), *L. edwardsi* (rat), and *P. larvata* (civet) (Table S4, Supporting Information).

(2) Explosive opening with damage to the flower (such as flower dropping), even though pollen may still be successfully transferred. This is done by birds *Pomatorhinus ruficollis* (Table S4, Supporting Information)

(3) Visitation of a previously opened flower, mostly done by rodents and civets (bees and other insects as

visitors were also counted but not used for data analysis in this study).

(4) Nectar robbing from an unopened flower, done by birds *P. ruficollis* (Table S4, Supporting Information).

(5) Bud feeding, done by squirrels and rats when only buds were available at both locations.

Based on the video tips, the behavioral groups (1) and (3) performed by rodents and civets were often mixed and some hard to distinguish from each other for each independent event because these animals often detected and opened different flowers or inflorescences using their snout or forelimbs during each visit, and flowers in each inflorescence or different inflorescences were often mature at different times (see also Kobayashi *et al.* 2015a, 2017). However, such repeated movement among different flowers may improve pollination efficiency (Kobayashi *et al.* 2015a, 2017). Therefore, I combined the behavioral groups (1) and (3) into one group, that is, explosive opening (Table 1). In addition, other visits behaviors to *M. sempervirens* vines (not related to flowers) were not analyzed in this study.

Assessing scatter-hoarding rodents as legitimate seed dispersers

I tracked the fate of individually tagged seeds over 5 years (2007–2011) at the above 2 locations used for flower visitors survey: Qingchengshan (squirrel present) and Banruosi (squirrel absent) (Table S2, Supporting Information). *M. sempervirens* seeds were collected from at least 1 of the 2 sites depending on seed availability each year. At each site, I annually labeled 150 or 90 seeds (total of 690 seeds for each site) 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). In November of each year, I placed 50 or 30 tagged seeds at each of 3 seed station (one-square-meter area) near the target vine at each location. After seed placement, the fate of the tagged seeds was monitored, each 1 or 2 weeks during the first 2 months. During each visit, I searched the area around each seed station (up to 10–50 m depending on seed removal) to retrieve removed seeds and record their fate. Caching sites made by rodents were marked using a numbered bamboo stick (15 cm × 1.5 cm). I rechecked the caches periodically in subsequent visits until cached seeds were recovered by animals. If a marked cache was removed, the area around the cache was also searched. When a cached seed was excavated and subsequently found re-cached, I measured the distance to its original seed source and to its previous cache. In the following spring, I also surveyed all seed stations and all pre-

viously found cache sites to determine the status of seed survival, or whether these surviving seeds were viable for germination.

In this study, I used the following binary response variables to assess seed dispersal efficiency by scatter-hoarding rodents: seeds were either removed or not from the source; cached or not after removal (primary caches); re-cached or not after recovery from primary caches (secondary caches); surviving in situ or not (at the source); surviving after hoarded at caches or not (the following spring). These seed dispersal data were analyzed using generalized linear mixed models (GLMM) with a logit link function and binomial error distribution. In the analysis, location (squirrel presence or absence) was treated as a fixed factor, and year as a random factor. In addition, GLMM with Gaussian error distribution was also used to test the effects of location (squirrel presence or absence) on dispersal distance (log transformed) of the hoarded seeds (primary caches) with year as a random factor. GLMMs were carried out in R 3.5.1 (R Core Team 2018) using the Package lme4 (Bates *et al.* 2013).

RESULTS

Identifying flower visitors and their behaviors

In this study, 7 mammals and birds were identified as explosive openers for *M. sempervirens* flowers, including 5 rodent species with 2 squirrel species *C. erythraeus* and *D. pernyi* only present at the Qingchengshan site (Table 1; Fig. 1). Rodents and civets were observed squeezing the keel with their noses or faces then feeding on nectar (Fig. S1, Supporting Information). Compared to fewer records as explosive openers by *Apodemus* mice and *Niviventer* rats at both locations, *L. edwardsi* and *P. larvata* were recorded more frequently as the main pollinators at the squirrel-absent site (Banruosi), while *C. erythraeus* squirrels were the main pollinators at the squirrel-present site (Qingchengshan). The only bird species *P. ruficollis* often acted as nectar robbers at the squirrel-absent site though it was also observed opening the flowers by stomping with legs (2 cases detected) (Table 1; Table S4, Supporting Information). Besides visiting flowers, *L. edwardsi* rats were detected up to 26 times feeding on buds particularly during earlier flowering seasons at the squirrel-absent site (Table 1).

Assessing scatter-hoarding rodents as legitimate seed dispersers

Seed fate pathways of *M. sempervirens* resulting from scatter-hoarding rodent activities indicate rodents acted as

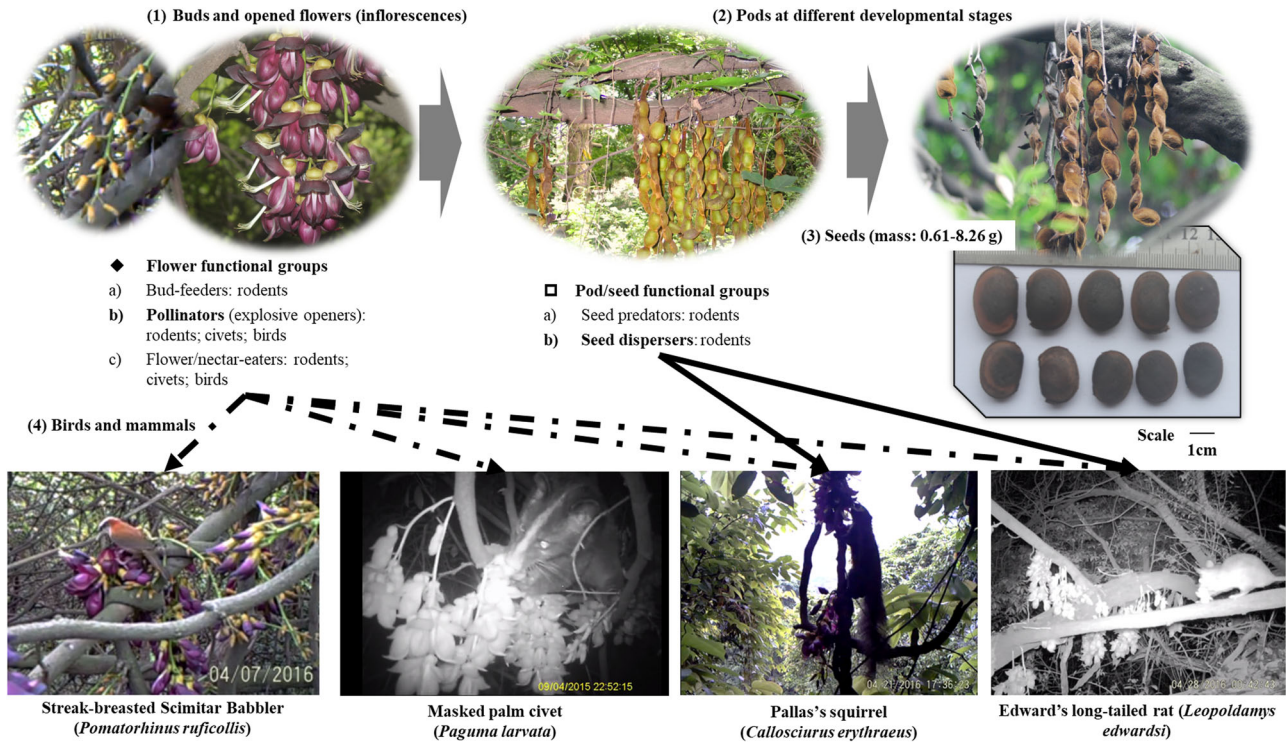


Figure 1 Flowers (inflorescences) and pods (seeds) of *Mucuna sempervirens* and their mammal/bird functional groups (see also detailed behaviors from video tips in supplement files) in the Dujiangyan forests, Southwest China. Based on our survey, the rodents and birds interacting with flowers and pods/seeds of *Mucuna* species can play up to 5 ecological roles (functional groups): bud feeders during early flowering seasons, flower/nectar feeders, pollinators during the flowering peak (1), and seed predators and dispersers during pod maturity (2) and after seedfall on the ground (3), and birds and mammals recorded using camera traps during flowering peak (4). Symbols: diamond, flower functional groups, and square, pod/seed functional groups. Dashed line, pollinators, and solid line, seed dispersers.

effective dispersers at both locations, but with slightly reduced dispersal (i.e. seed dispersal rate in primary caches) and more seeds remaining in situ at the squirrel-absent site (Fig. 2). In this study, over 70% of the tagged seeds were relocated at least once, but over 40% of the tagged seeds were missing but may be dispersed outside of the search area (Fig. 2). After seed placement, over 70% of the tagged seeds were removed by scatter-hoarding rodents at both sites ($z = -0.42$, $P = 0.673$), but over 25% of them were not harvested and then survived until the next spring at the squirrel-absent site (compared to 2.72% at the squirrel-present site, $z = -11.88$, $P < 0.001$; Fig. 2). However, a slightly higher proportion of the tagged seeds were hoarded at the squirrel-present site (29.4%) than that at the squirrel-absent site (25.7%) ($z = -5.03$, $P < 0.001$; Fig. 2). Additionally, several seeds from primary caches and secondary caches were recovered and then moved into higher-order caches. Finally, a small portion of tagged seeds (1–3.5%) survived and

germinated after being cached at both sites ($z = -1.06$, $P = 0.291$; Fig. 2). All caches relocated during the study were found to contain only one seed. Mean dispersal distance of the hoarded seeds in primary caches was similar between both sites (squirrel-absent site, 11.5 ± 11.1 m; squirrel-present site, 11.5 ± 12.3 m; $t = 3.11$, $P > 0.05$; Fig. 3).

DISCUSSION

This study provides sound support for the hypothesis that scatter-hoarding rodents could act as double mutualists for the same plant *M. sempervirens*. In this study, I not only demonstrate several rodents (here *L. edwardsi* and *C. erythraeus*) as main pollinators by explosive opening to *M. sempervirens* flowers, but also provide the first evidence that these rodents could act as legitimate seed dispersers via scatter-hoarding in this world-wide plant genus. More importantly, this is the first study to represent

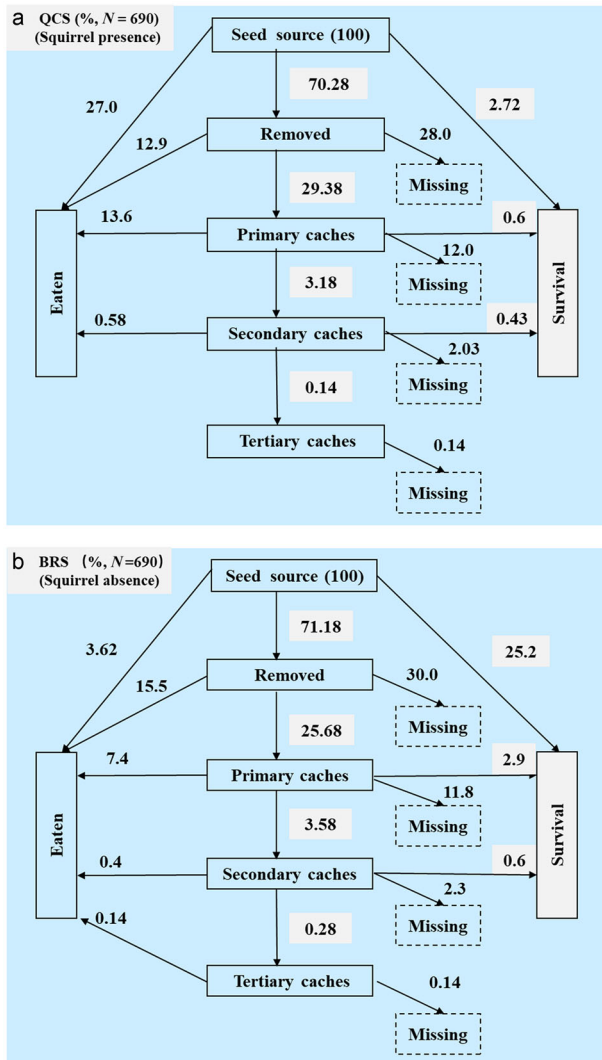


Figure 2 Seed fate pathways of *Mucuna sempervirens* by scatter-hoarding rodents over 5 years (pooled, 2007–2011) at 2 locations (QCS, Qingchengshan with squirrel presence; BRS, Banruosi without squirrel presence) in the Dujiangyan forests, Southwest China. Key processes of seed fate pathways are shown in solid boxes (missing parts in dashed boxes), and the numbers in each pathway indicate percentages (seed fate).

a rare double mutualism on the Eurasian mainland (see Fuster *et al.* 2019). Moreover, the double mutualism phenomena between *Mucuna* species and scatter-hoarding rodents may be rare among dry-seeded plants producing zygomorphic flowers but may be pervasive across the species distribution of *Mucuna* subg. *Macrocarpa* at least in the (sub)tropical Asia (Table S3, Supporting Information).

Scatter-hoarding rodents as double mutualists in *Mucuna*

Though some mammal or bird species have been documented as legitimate pollinators for related *Mucuna* species on different continents (Kobayashi 2018), the key question regarding to double mutualism is whether and how at least some pollinators should also act as legitimate seed dispersers for the same *Mucuna* species. However, other known mammals (e.g. bats, civets, primates) or birds as key pollinators may not use dry seeds as a food resource and thus cannot serve as legitimate seed dispersers for the dispersal of dry seeds produced by the same *Mucuna* species. Recently, some studies about *Mucuna* pollination have demonstrated several rodent species (squirrels and rats in particular) as legitimate pollinators for at least 4 *Mucuna* species in (sub)tropical Asia (Table S3, Supporting Information). Considering that large proportions (over 70%) of *M. sempervirens* seeds were removed and then hoarded (successfully dispersed) by scatter-hoarding rodents (Fig. 2), I expect that scatter-hoarding rodents as double mutualists may be widely represented across the *Mucuna* distribution (mostly *Mucuna* subg. *Macrocarpa*) in (sub)tropical Asia, because some rodent species have been identified as main pollinators for at least 4 *Mucuna* species in different parts of (sub)tropical Asia (Japan, South China and Thailand) (Table S3, Supporting Information).

In this study, I found that scatter-hoarding rodents can use *M. sempervirens* flowers (buds, flower pieces, and nectars) and seeds as food sources during different seasons at different sites. Thus, scatter-hoarding rodents can play up to 5 ecological roles (functional groups) when they interact with flowers and seeds of *Mucuna* species: bud feeders early in the flowering season, flower/nectar feeders and pollinators during the flowering peak, and seed predators and dispersers during pod maturity and after seedfall on the ground (Fig. 1). The multiple roles performed by scatter-hoarding rodents indicate that they are essential for reproductive success and population persistence in these *Mucuna* species. In general, scatter-hoarding rodents are often generalists by using a diverse range of plant species or different plant parts as food resources. At the studied sites, scatter-hoarding rodents are found as important seed dispersers for over 30 seed species (Xiao *et al.* 2003, 2005; Lai *et al.* 2014; Zhishu Xiao, unpublished data). Seeds from most *Mucuna* species (e.g. *M. sempervirens* in this study) are relatively large (mean, 4.4 g) but with hard, woody seed coats (physical defense) and high content of L-Dopa (chem-

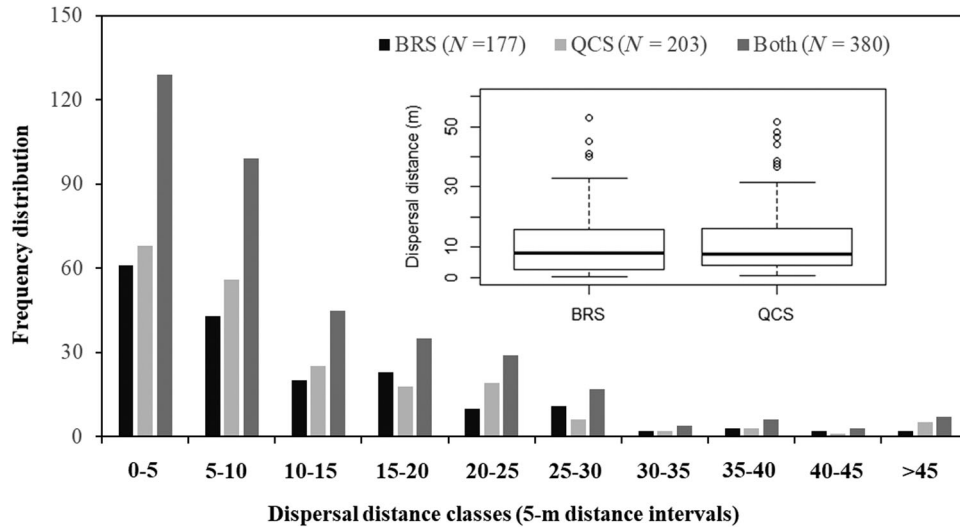


Figure 3 Dispersal distance (m) and its frequency distribution of the dispersed seeds in primary caches of *Mucuna sempervirens* by scatter-hoarding rodents over 5 years (2007–2011) at 2 locations (QCS, Qingchengshan with squirrel presence; BRS, Banruosi without squirrel presence; Both, both sites) in the Dujiangyan forests, Southwest China.

ical defense). Similar to those detected in other large-seeded species (e.g. Fagaceae species, Xiao *et al.* 2005), these seed traits from these *Mucuna* species may not only be effective at reducing seed predation, but also at encouraging scatter-hoarding rodents to disperse seeds. In this way, scatter-hoarding rodents would act as obligatory dispersal vectors for these *Mucuna* species, where water dispersal may be unusual and gravitational dispersal may be limited for population colonization in forest ecosystems. Therefore, the double mutualism between these *Mucuna* species and scatter-hoarding rodents provide clear evidence for one main hypothesis proposed by Fuster *et al.* (2019): double mutualisms would be more likely prevalent in (sub)tropical communities, where many generalized species can extend trophic niches in diverse ways.

Recently, Moura *et al.* (2016b) separated one new subgenus, *Mucuna* subg. *Macrocarpa*, from *Mucuna* subg. *Mucuna*, including *M. sempervirens*, *M. birdwoodiana*, and *M. macrocarpa* only distributed in eastern and southeastern Asia. According to my study and other related studies (Table S3, Supporting Information), it is evident that the known legitimate pollinators of these species from *M. subg. Macrocarpa* are mostly non-bat mammals, while bats or birds had been recorded as the legitimate pollinators for other *Mucuna* species (Kobayashi 2018). Therefore, the double mutualism phenomena between these species from *M. subg. Macrocarpa* and scatter-hoarding rodents may also provide additional evidence

for phylogeny and biogeography of this world-wide plant genus.

Implications for double mutualism conservation in *Mucuna* species

As discussed before, explosive opening is required for reproductive success in most *Mucuna* species. In this study, I have shown that explosive opening and subsequent pollination of *M. sempervirens* flowers by different rodents would be very important for annual seed production at each location, where the main rodent pollinators were not the same species. My camera-trap survey confirmed that *C. erythraeus* squirrels were the main rodent pollinators at the squirrel-present site, while *L. edwardsi* rats were the main rodent pollinators at the squirrel-absent site (Table 1 and Table S1, Supporting Information). However, *L. edwardsi* rats also had more records for bud feeding, indicating food resources for this rodent species may be limited at the squirrel-absent site compared to that at the squirrel-present site. However, frequent visitation but with higher-efficiency pollination by *P. larvata* (see also Kobayashi *et al.* 2018b) could improve lower-efficiency pollination provided by *L. edwardsi* and other rodents at the squirrel-absent site. Based on our survey before, *P. larvata* also has higher occupancy at the squirrel-present site (Xiao *et al.* 2014a). However, some mammal pollinators including primates

(e.g. *Macaca* spp.), martens, civets, and bats may be locally extirpated in some highly disturbed areas; this can lead to pollination failure in some locations along the *Mucuna* distribution if rodent species are not able to compensate for extirpated pollinator species. Therefore, pollination and seed production of *Mucuna* species would be ensured if a diverse community of legitimate pollinators co-exists, especially in human-disturbed regions.

Based on my seed-dispersal survey over 5 years, I found that scatter-hoarding rodents provide dispersal services for *M. sempervirens* at both locations with more than 70% of the tagged seeds removed and over 25% of them hoarded in one-seed caches. More importantly, I did find that a portion of the hoarded seeds survived until seed germination in the following spring, and dispersal distance of the hoarded seeds in primary caches was also similar between both locations. This indicates that other scatter-hoarding rodents could act as legitimate seed dispersers as done by scatter-hoarding squirrels in natural forest settings. However, I also observed a reduction in dispersal services in human-disturbed habitats which had more seeds remaining in situ and fewer seeds dispersed and cached. Consequently, seed dispersal efficiency of *Mucuna* species may be limited in some highly disturbed areas. Furthermore, seed predation may be more intensive when food resources are scarce in human-disturbed landscapes (McConkey *et al.* 2012). Further research should assess whether seed dispersal efficiency by scatter-hoarding rodents is reduced across human-disturbed landscapes when compared to undisturbed habitats.

In China and, maybe, other parts of (sub)tropical Asia, *M. sempervirens* and other *Mucuna* populations may have been reduced since these plants are often used as medicine or ornamental plants by local people, and currently small populations are also present in many botanical gardens, arboretums, and backyards. Animal mutualists and their pollination/dispersal services may vary greatly across different geographic regions (e.g. *M. macrocarpa*, Kobayashi *et al.* 2020), so more specialized mutualist populations could be significantly reduced or even lost in many highly disturbed habitats (Corlett 2017; Fedriani *et al.* 2020). In fact, *M. sempervirens* populations in some botanical gardens are found to produce massive flowers but no pods, indicating mutualism disruption in these ex situ conservation sites (Chen *et al.* 2012; Zhishu Xiao, unpublished data). Through cascading effects, the cryptic threatened ecological functions such as pollination and seed dispersal may have significant impacts on human-dominated ecosystems (McConkey *et al.* 2012;

Regan *et al.* 2015). Therefore, knowledge about mutualism between *Mucuna* species and their animal partners should be essential for ex situ conservation in botanical gardens. Based on the above discussions, I propose 2 key conservation priorities for this rare double mutualism in *Mucuna*-animal systems: (1) in situ conservation of *Mucuna* species would be more reliable than ex situ conservation (e.g. botanical gardens) where fewer generalist pollinators and seed dispersers may be present; and (2) ex situ conservation should be conducted in areas with rich mutualist communities for both pollination and seed dispersal services.

CONCLUSIONS

This study demonstrates that in addition to acting as main pollinators, scatter-hoarding rodents could act as legitimate dispersal vectors for the same *Mucuna* species producing large seeds, although dispersal services were slightly reduced at squirrel-absent site. Though I have limited samples from 2 *M. sempervirens* locations, my data indicate a clear relationship of double mutualism in those rodent-pollinated *Mucuna* species, and the dual roles of scatter-hoarding rodents as key pollinators and seed dispersers may be essential for the double mutualism conservation in these *Mucuna* populations in human-disturbed landscapes. For a better understanding of the ecology and evolution of double mutualisms worldwide, more joint research about pollination efficiency and seed dispersal effectiveness by animal mutualists should be conducted to further understand how the *Mucuna*-animal system varies in space and time. Such detailed research would benefit the conservation of this unique system, the animal partners, and the fragile ecosystems where they persist.

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REFERENCES

Agostini K, Sazima M, Sazima I (2006). Bird pollination of explosive flowers while foraging for nectar and caterpillars. *Biotropica* **38**, 674–8.

- Bates D, Maechler M, Bolker B, Walker S (2013). lme4: Linear MixedEffects Models Using Eigen and S4. Available from URL: <http://CRAN.R-project.org/package=lme4>
- Chang G, Zhang Z (2011). Differences in hoarding behaviors among six sympatric rodent species on seeds of oil tea (*Camellia oleifera*) in Southwest China. *Acta Oecologica* **37**, 165–9.
- Chen G, Zhang R, Dong K, Gong W, Ma Y (2012). Scented nectar of *Mucuna sempervirens* and its ecological function. *Biodiversity Science* **20**, 360–7. (In Chinese with English summary.)
- Corlett RT (2017). Frugivory and seed dispersal by vertebrates in tropical and subtropical Asia: An update. *Global Ecology and Conservation* **11**, 1–22.
- Dong YC (2012). Geographic distribution and application of *Mucuna sempervirens* Hems. *Journal of Anhui Agriculture Science* **40**, 2633–5. (In Chinese.)
- Fedriani JM, Ayllon D, Wiegand T, Grimm V (2020). Intertwined effects of defaunation, increased tree mortality and density compensation on seed dispersal. *Ecography* **43**, 1352–63.
- Fuster F, Traveset A (2019). Evidence for a double mutualistic interaction between a lizard and a Mediterranean gymnosperm, *Ephedra fragilis*. *AoB Plants* **11**, plz001.
- Fuster F, Traveset A (2020). Importance of intraspecific variation in the pollination and seed dispersal functions of a double mutualist animal species. *Oikos* **129**, 106–16.
- Fuster F, Kaiser-Bunbury C, Olesen JM, Traveset A (2019). Global patterns of the double mutualism phenomenon. *Ecography* **42**, 826–35.
- Gomes VGN, Quirino ZGM, Machado IC (2014). Pollination and seed dispersal of *Melocactus ernestii* Vaupel subsp. *ernestii* (Cactaceae) by lizards: an example of double mutualism. *Plant Biology* **16**, 315–22.
- Gu H, Zhao Q, Zhang Z (2017). Does scatter-hoarding of seeds benefit cache owners or pilferers. *Integrative Zoology* **12**, 477–88.
- Hansen DM, Mueller CB (2009). Reproductive ecology of the endangered enigmatic Mauritian endemic *Roussea simplex* (Rousseaceae). *International Journal of Plant Sciences* **170**, 42–52.
- Herrera CM, Pellmyr O (2002). *Plant-Animal Interactions: An Evolutionary Approach*. Blackwell Science, Hoboken, NJ.
- Hopkins HCF, Hopkins MJG (1993). Rediscovery of *Mucuna macropoda* (Leguminosae: Papilionoideae), and its pollination by bats in Papua New Guinea. *Kew Bulletin* **48**, 297–305.
- Jiang M, Cao L, Xiao Z, Guo C (2010). Frugivorous birds and its impacts on seed dispersal of wild cherry (*Prunus pseudocerasus*) in a Dujiangyan forest, China. *Chinese Journal of Zoology* **45**, 27–34. (In Chinese with English summary.)
- Kelly D, Ladley JJ, Robertson AW (2004). Is dispersal easier than pollination? Two tests in New Zealand Loranthaceae. *New Zealand Journal of Botany* **42**, 89–103.
- Kobayashi S (2018). Regional differences in mammalian pollinators of *Mucuna macrocarpa* (Leguminosae): A review. *Tropical Natural History* **18**, 135–45.
- Kobayashi S, Denda T, Mashiba S, Iwamoto T, Doi T, Izawa M (2015a). Pollination partners of *Mucuna macrocarpa* (Fabaceae) at the northern limit of its range. *Plant Species Biology* **30**, 272–8.
- Kobayashi S, Denda T, Mashiba S, Doi T, Izawa M (2015b). Appearance of new relationship between *Mucuna macrocarpa* (Fabaceae) and Japanese macaque as pollination partner: Indirect effect of afforestation policy. *Primate Research* **31**, 39–47.
- Kobayashi S, Denda T, Liao CC, Wu SH, Lin YH, Izawa M (2017). Squirrel pollination of *Mucuna macrocarpa* (Fabaceae) in Taiwan. *Journal of Mammalogy* **98**, 533–41.
- Kobayashi S, Denda T, Liao CC, Lin YH, Wu SH, Izawa M (2018a). Floral traits of mammal-pollinated *Mucuna macrocarpa* (Fabaceae): Implications for generalist-like pollination systems. *Ecology and Evolution* **8**, 8607–15.
- Kobayashi S, Denda T, Liao CC, Lin YH, Liu TW, Izawa M (2018b). Comparison of visitors and pollinators of *Mucuna macrocarpa* between urban and forest environments. *Mammal Study* **43**, 219–28.
- Kobayashi S, Hirose E, Denda T, Izawa M (2018c). Who can open the flower? Assessment of the flower opening force of mammal-pollinated *Mucuna macrocarpa*. *Plant Species Biology* **33**, 312–6.
- Kobayashi S, Gale SWT, Denda T, Izawa M (2019a). Civet pollination in *Mucuna birdwoodiana* (Fabaceae: Papilionoideae). *Plant Ecology* **220**, 457–66.

- Kobayashi S, Denda T, Placksanoi J *et al.* (2019b). The pollination system of the widely distributed mammal-pollinated *Mucuna macrocarpa* (Fabaceae) in the tropics. *Ecology and Evolution* **9**, 6276–86.
- Kobayashi S, Denda T, Liao CC *et al.* (2020). Effects of different pollinators and herbivores on the fruit set height of the mammal-pollinated tree-climbing vine *Mucuna macrocarpa*. *Journal of Forest Research* **25**, 315–21.
- Kobayashi S, Gale SW, Denda T, Izawa M (2021). Rat- and bat-pollination of *Mucuna championii* (Fabaceae) in Hong Kong. *Plant Species Biology* **36**, 84–93.
- Kuprewicz EK (2013). Mammal abundances and seed traits control the seed dispersal and predation roles of terrestrial mammals in a Costa Rican forest. *Biotropica* **45**, 333–42.
- Kuprewicz EK, Garcia-Robledo C (2010). Mammal and insect predation of chemically and structurally defended *Mucuna holtonii* (Fabaceae) seeds in a Costa Rican rain forest. *Journal of Tropical Ecology* **26**, 263–9.
- Lai X, Guo C, Xiao Z (2014). Trait-mediated seed predation, dispersal and survival among frugivore-dispersed plants in a fragmented subtropical forest, Southwest China. *Integrative Zoology* **9**, 246–54.
- Lewis G, Schirire B, Mackinder B, Lock B (2005). *Legumes of the World*. Kew Royal Botanic Garden, London, pp. 55–510.
- Li H, Tang L, Jia C *et al.* (2020). The functional roles of species in metacommunities, as revealed by metanetwork analyses of bird-plant frugivory networks. *Ecology Letters* **23**, 1252–62.
- McConkey KR, Prasad S, Corlett RT *et al.* (2012). Seed dispersal in changing landscapes. *Biological Conservation* **146**, 1–13.
- Moura TM, Vatanparast M, Tozzi AMGA *et al.* (2016a). A molecular phylogeny and new infrageneric classification of *Mucuna* Adans. (Leguminosae-Papilionoideae) including insights from morphology and hypotheses about biogeography. *International Journal of Plant Sciences* **177**, 76–89.
- Moura TM, Wilmot-Dear CM, Vatanparast M, Fortuna-Perez AP, Tozzi AMGA, Lewis GP (2016b) A new infrageneric classification of *Mucuna* (Leguminosae-Papilionoideae): Supported by morphology, molecular phylogeny and biogeography. *Systematic Botany* **41**, 606–16.
- Nakamoto A, Kinjo K, Izawa M (2009). The role of Orii's flying-fox (*Pteropus dasymallus inopinatus*) as a pollinator and a seed disperser on Okinawa-jima Island, the Ryukyu Archipelago, Japan. *Ecological Research* **24**, 405–14.
- Olesen JM, Damgaard CF, Fuster F *et al.* (2018). Disclosing the double mutualist role of birds on Galapagos. *Scientific Reports* **8**, 57.
- Ollerton J, Winfree R, Tarrant S (2011). How many flowering plants are pollinated by animals? *Oikos* **120**, 321–6.
- R Development Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. Available from URL: <http://www.R-project.org/>
- Regan EC, Santini L, Ingwall-King L *et al.* (2015). Global trends in the status of bird and mammal pollinators. *Conservation Letters* **8**, 397–403.
- Sa R, Wilmot-Dear CM (2010). *Mucuna*. In: Wu ZY, Raven PH, Hong DY, eds. *Flora of China 10*. Fabaceae. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, pp. 207–8.
- Toyama C, Kobayashi S, Denda T, Nakamoto A, Izawa M (2012). Feeding behavior of the Orii's flying-fox, *Pteropus dasymallus inopinatus*, on *Mucuna macrocarpa* and related explosive opening of petals, on Okinawajima Island in the Ryukyu Archipelago, Japan. *Mammal Study* **37**, 205–12.
- van der Pijl L (1941). Flagelliflory and cauliflory as adaptations to bats in *Mucuna* and other plants. *Annals of the Botanic Gardens, Buitenzorg* **51**, 83–93.
- Xiao Z, Zhang Z, Wang Y (2003). Observations on tree seed selection and caching by Edward's long-tailed rat (*Leopoldamys edwardsi*). *Acta Theriologica Sinica* **23**, 208–13. (In Chinese with English summary.)
- Xiao Z, Zhang Z (2004). Small mammals consuming tree seeds in Dujiangyan forest, China. *Acta Theriologica Sinica*. **24**, 121–4. (In Chinese with English summary.)
- Xiao Z, Zhang Z, Wang Y (2005). Effects of seed size on dispersal distance in five rodent-dispersed fagaceous species. *Acta Oecologica* **28**, 221–9.
- Xiao Z, Jansen PA, Zhang Z (2006). Using seed-tagging methods for assessing post-dispersal seed fate in rodent-dispersed trees. *Forest Ecology and Management* **223**, 18–23.

- Xiao Z, Chang, G, Zhang Z (2008). Testing the high-tannin hypothesis with scatter-hoarding rodents: experimental and field evidence. *Animal Behaviour* **75**, 1235–41.
- Xiao Z, Gao, X, Jiang MM, Zhang Z (2009). Behavioral adaptation of Pallas's squirrels to germination schedule and tannins in acorns. *Behavioral Ecology* **20**, 1050–5.
- Xiao Z, Wang X, Huang X (2014a). Mammal and bird diversity in Qingchengshan Forest Park, Southwest China: data from camera traps. *Biodiversity Science* **22**, 788–93. (In Chinese with English summary.)
- Xiao Z, Wang X, Li X (2014b). An introduction to CameraData: an online database of wildlife camera trap data. *Biodiversity Science* **22**, 712–6. (In Chinese with English summary.)
- Xiao Z, Zhang Z (2016). Contrasting patterns of short-term indirect seed-seed interactions mediated by scatter-hoarding rodents. *Journal of Animal Ecology* **85**, 1370–7.
- Yang X, Zhou R (1992). Studies on chemotaxonomy of *Mucuna* and *Stizolobium*. *Journal of China Pharmaceutical University* **23**, 74–6. (In Chinese with English summary.)
- Zhou R, Yang X, Tang Z (1988). Studies on L-Dopa resource plants of Chinese *Mucuna*. *Zhongyaocai* **11**, 22–4. (In Chinese.)
- Zies P (1999). Batty about sea-beans. *The Drifting Seed* **5**, 6–7.

SUPPLEMENTARY MATERIALS

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Monitoring mammal and bird visitors to *Mucuna sempervirens* flowers at 2 locations (Qingchengshan with squirrel presence; Banruosi without squirrel presence) in the Dujiangyan forests, Southwest China

Table S2 Sampling the fate of individually-marked seeds of *Mucuna sempervirens* over 5 years (2007–2011) at 2 locations (Qingchengshan with squirrel presence; Banruosi without squirrel presence) in the Dujiangyan forests, Southwest China

Table S3 Double mutualisms for pollination and seed dispersal of some *Mucuna* species (Fabaceae) identified from literature survey and this study. Rodent species as potential double mutualists are shown in **bold**.

Table S4 Video tips for identifying mammals or birds visiting *Mucuna sempervirens* flowers and their foraging behaviors using camera traps.

Figure S1 Flowering phenology with 4 main stages of *Mucuna sempervirens* at 2 locations (Qingchengshan with squirrel presence; Banruosi without squirrel presence) in the Dujiangyan forests, Southwest China Stages.

Supplementary videos.

Supplementary videos.

Supplementary videos.

Supplementary videos.

Supplementary videos.

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