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Annotating PDFs using Adobe Reader XI

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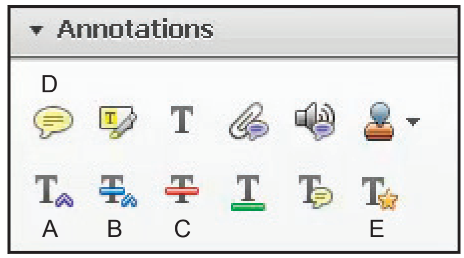
1. Update to Adobe Reader XI

The screen images in this document were captured on a Windows PC running Adobe Reader XI. Editing of DJS proofs requires the use of Acrobat or Reader XI or higher. At the time of this writing, Adobe Reader XI is freely available and can be downloaded from http://get.adobe.com/reader/

2. What are eProofs?

eProof files are self-contained PDF documents for viewing on-screen and for printing. They contain all appropriate formatting and fonts to ensure correct rendering on-screen and when printing hardcopy. DJS sends eProofs that can be viewed, annotated, and printed using the free version of Acrobat Reader XI (or higher).

3. Comment & Markup toolbar functionality



A. Insert Text tool
B. Replace Text tool
C. Delete Text tool
D. Sticky Note tool
E. Text Correction Markup tool

A. Show the Comment & Markup toolbar

The Comment & Markup toolbar doesn't appear by default. Do one of the following:

- Select View > Comment > Annotations.
- Click the Comment button in the Task toolbar.

Note: If you've tried these steps and the Annotation Tools do not appear, make sure you have updated to version XI or higher.

B. Select a commenting or markup tool from the Annotations window.

Note: After an initial comment is made, the tool changes back to the Select tool so that the comment can be moved, resized, or edited. (The Pencil, Highlight Text, and Line tools stay selected.)

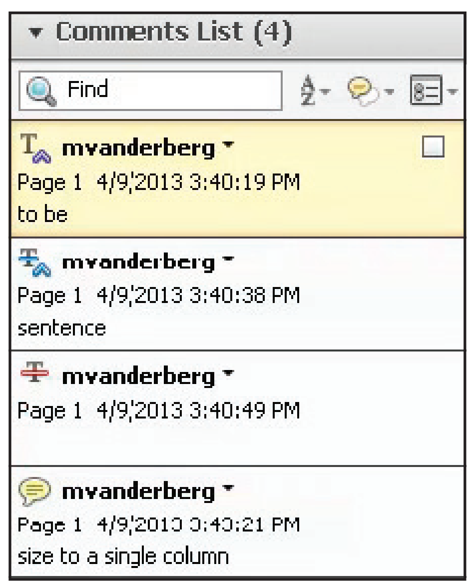
C. Keep a commenting tool selected

Multiple comments can be added without reselecting the tool. Select the tool to use (but don't use it yet).

- Right Click on the tool.
- Select Keep Tool Selected.

4. Using the comment and markup tools

To *insert*, *delete*, or *replace* text, use the corresponding tool. Select the tool, then select the text with the cursor (or simply position it) and begin typing. A pop-up note will appear based upon the modification (e.g., inserted text, replacement text, etc.). Use the Properties bar to format text in pop-up notes. A pop-up note can be minimized by selecting the ⊟ button inside it. A color-coded ⅄ symbol will remain behind to indicate where your comment was inserted, and the comment will be visible in the Comments List.

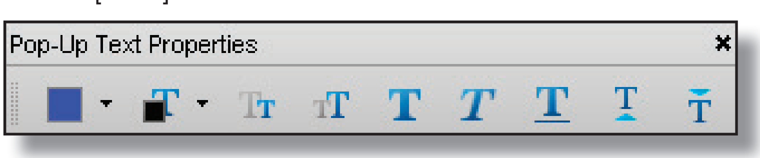


5. The Properties bar

The Properties bar can be used to add formatting such as bold or italics to the text in your comments.

To view the Properties bar, do one of the following:

- Right-click the toolbar area; choose Properties Bar.
- Press [Ctrl-E]



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6. Inserting symbols or special characters

An ‘insert symbol’ feature is not available for annotations, and copying/pasting symbols or non-keyboard characters from Microsoft Word does not always work. Use angle brackets < > to indicate these special characters (e.g., <alpha>, <beta>).

7. Editing near watermarks and hyperlinked text

eProof documents often contain watermarks and/or hyperlinked text. Selecting characters near these items can be difficult using the mouse alone. To edit an eProof which contains text in these areas, do the following:

- Without selecting the watermark or hyperlink, place the cursor near the area for editing.
- Use the arrow keys to move the cursor beside the text to be edited.
- Hold down the shift key while simultaneously using arrow keys to select the block of text, if necessary.
- Insert, replace, or delete text, as needed.

8. Summary of main functions

A. Insert text - Use Insert Text tool (position cursor and begin typing)
B. Replace text - Use Replace Text tool (select text and begin typing)
C. Delete text - Use Strikethrough Text tool (select text and press delete key)
Note: The Text Correction Markup tool combines the functions of all three tools.
D. Sticky Note - Use Sticky Note tool to add comments not related to text correction.

9. Reviewing changes

To review all changes, do the following:

- Click the Comments button to reveal the comment tools
- Click the triangle next to Comments List (if not already visible)

Note: *Selecting a correction in the list will highlight the corresponding item in the document, and vice versa.*

10. Still have questions?

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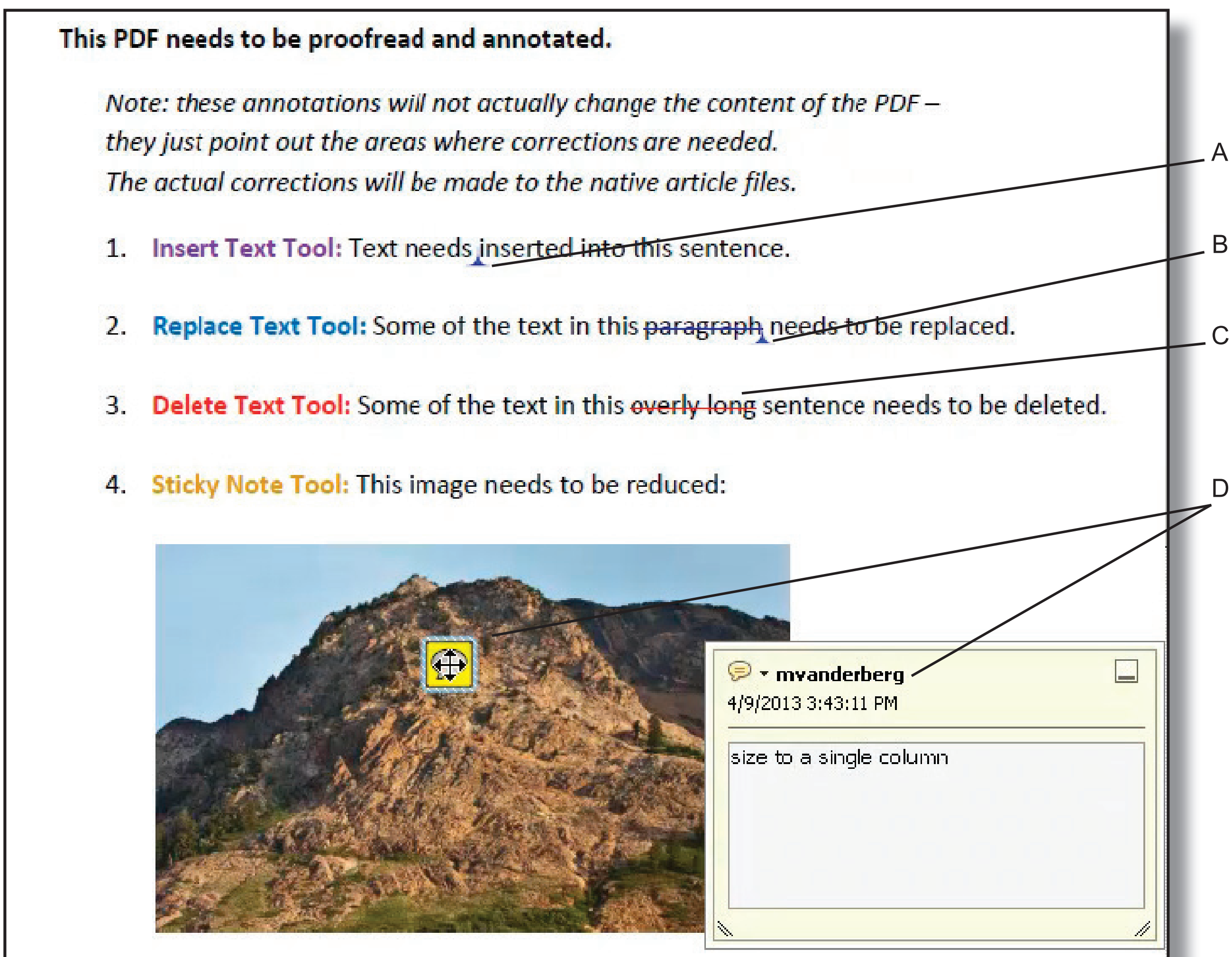

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Insect pollination and self-incompatibility in edible and/or medicinal crops in southwestern China, a global hotspot of biodiversity[1]

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• An increasing global demand for food, coupled with the widespread decline of pollinator diversity, remains an international concern in agriculture and genetic conservation. In particular, there are large gaps in the study of the pollination of economically important and traditionally grown species in China. Many plant species grown in China are both edible and used medicinally. The country retains extensive written records of agricultural and apicultural practices, facilitating contemporary studies of some important taxa. Here, we focus on Yunnan in southwestern China, a mega-biodiversity hotspot for medicinal/food plants. We used plant and insect taxa as model systems to understand the patterns and consequences of pollinator deficit to crops. We identified several gaps and limitations in research on the pollination ecology and breeding systems of domesticated taxa and their wild relatives in Yunnan and asked the following questions: (1) What is known about pollination systems of edible and medicinal plants in Yunnan? (2) What are the most important pollinators of *Codonopsis subglobosa* (Campanulaceae)? (3) How important are native pollinator species for maximizing yield in Chinese crops compared with the introduced *Apis mellifera*? We found that some crops that require cross-pollination now depend exclusively on hand pollination. Three domesticated crops are dependent primarily on the native but semidomesticated *Apis cerana* and the introduced *A. mellifera.* Other species of wild pollinators often play important roles for certain specialty crops (e.g., *Vespa velutina* pollinates *Codonopsis subglobosa*). We propose a more systematic and comprehensive approach to applied research in the future.

Key words: breeding system; crop; domestication; food; honeybee; medicinal plants; pollinators; southwestern China; self-incompatibility; yield.

Animal pollinators are needed for the reproduction of 90% of angiosperm species and at least 75% of food crops (Free, 1970; Ollerton et al., 2011). Humans are estimated to owe one in three bites of food to insect pollination (Holden, 2006). Approximately 90% of edible vitamin C is produced by crop species dependent fully or partially on animal pollinators. These insect-pollinated crops also produce essential micronutrients such as lycopene (Eilers et al., 2011). Using a database that compiled information from 200 countries, Klein et al. (2007) found that fruit, vegetable, or seed production from 87 of the leading global crops depends on animal pollination. The total economic value of pollination worldwide amounted to approximately $215 billion, representing 9.5% of the value of the world's agricultural production for food in 2005 (Gallai et al., 2009). The human population worldwide is projected to increase by an additional 2.4 billion by the year 2050 (Godfray et al., 2010), so global crop production at that time will need to double compared with 2005 (Tilman et al., 2011). As global food demand increases, so will human dependence on insect pollinators. However, the global widespread decline of pollinator populations and diversity continues to cause international concern in commercial agriculture and plant conservation (Klein et al., 2007; Potts et al., 2010; Burkle et al., 2013).

Evidence suggests that pollinator decline is a global trend contributing to pollination deficit/limitation in both wild plant species and domesticated crops (Thomann et al., 2013). However, data used to assess and address this phenomenon are uneven because most of the research, to date, comes primarily from Europe, the United States, and Canada (Klein et al., 2007; Potts et al., 2010; Garibaldi et al., 2013; Kennedy et al., 2013). Data from developing countries in the tropical Americas, sub-Saharan Africa, and temperate and tropical Asia remains deficient even though these continents still retain the highest diversity of native and domesticated plant species. Unfortunately, the problem is further aggravated by the fact that these regions also have the largest populations to feed. Global food security is and will be a huge challenge for humans in the 21st century, especially for countries with the largest populations such as China (Godfray et al., 2010).

China has the largest population in the world, and the second-biggest economy, which continues to grow. While China is also one of the largest agricultural countries, its increasing population and living standards demand an exponential boost in agricultural products. In 2011, China exceeded the United

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States as the largest food importer in the world. According to the global trade data from the World Trade Organization (WTO), China imported $144.7 billion in food in 2011. While soybeans represented the major import, China also imported large quantities of insect-pollinated palm and rapeseed oils (FAOSTAT, 2014). Increasing the yield of pollinator-mediated crops in China is a recent concern and national policies backed by research are lacking (Liu et al., 2011a).

The flora of China is extremely diverse with 31 000 species of vascular plants. This diversity is attributed to variations in climate, latitude, topography, and geological history (Li and Pritchard, 2009). Approximately two thirds of the vascular plant species are found in southwestern China (regarded as a global hotspot of biodiversity) with the highest diversity of species and endemism of all temperate floras (Myers et al., 2000; Yang et al., 2004). Therefore, it is no surprise that China is also a center of crop domestication (Vavilov, 1992). Of approximately 1500 crop plants cultivated worldwide, 300 crop species originated and/or were domesticated and/or underwent differentiation in China (Huang, 2011).

Some economic botanists and ethnobotanists identify southwestern China as regions of aboriginal domestication of tea, buckwheat, and several important medicinal plants still used extensively in traditional medicine, including notoginseng (*Panax notoginseng* (Burkill) F.H. Chen ex C.Y. Wu & K.M. Feng; Araliaceae; Yang et al., 2004; López-Pujol et al., 2006). Medicinal plants continue to play prominent and traditional roles in the general health of the Chinese populations (e.g., ginger, *Zingiber officinale* Roscoe, Zingiberaceae; ginseng, *Panax ginseng* C.A. Mey) because these same species are also used as spices, vegetables, and diet supplements (Gao et al., 2005; Yuan et al., 2010). The propagation of a number of edible-medicinal species began in the 1970s (e.g., *Gastrodia elata* Blume [Orchidaceae] in Yunnan; Zhou et al., 1987). China has been growing at an average annual gross domestic product (GDP) growth rate of about 10% for last decade, which continues to exert considerable pressure on all indigenous plant resources and their genetics (López-Pujol et al., 2006; Li and Pritchard, 2009), stimulating the domestication of edible/medicinal species once gathered from the wild. To fulfill this demand, we need more research on breeding and pollination systems of both domesticated and wild species.

In addition, China's long history of sustainable agriculture includes apiculture (beekeeping). Of the 10–12 honeybee species described (sensu Michener, 2000), Chinese agriculture employs the semidomesticated *Apis cerana* and the domesticated and commercial *A. mellifera* (Apidae). They are regarded as the most important pollinators of crop plants in China, but *A. cerana* is the indigenous species and probably evolved with the Sino-flora since the mid-Tertiary (Smith et al., 2000; You et al., 2005).

In our review here, we focus on edible and medicinal crops of Yunnan in southwestern China because species diversity is extremely high in this region presumed to be a center of origin/variation for economically important plants used for food and medicine. Our long-term goals are to study these plant and insect taxa to understand the patterns and consequences of pollinator deficit. Here we address the following questions to better understand current limitations in our knowledge of these systems: (1) What is known about pollination systems of edible and medicinal plants in southwestern China? (2) What is the breeding system and pollination biology of *Codonopsis subglobosa* W.W. Smith (Campanulaceae), a cultivated but nondomesticated edible-medicinal species? (3) How is the native pollinator *Apis cerana* used in southwestern China, and how does this compare with the use of the introduced *A. mellifera*?

MATERIALS AND METHODS

Study area—We focused on a select number of crop plants in southwestern China grown in Yunnan Province (Fig. 1) for two reasons. First, about 25 ethnic minorities are found in Yunnan. Such diversity resulted in a long, multicultural history of agricultural practices and the exploitation of the native flora for food, medicine, and more recently, for tourism (Yang et al., 2004). While the most economically important crops in Yunnan are rapeseed (*Brassica campestris* L., *B. napus* L., and their hybrids; Brassicaceae), sugarcane (*Saccharum officinarum* L.; Poaceae), and commercial tealeaf (*Camellia sinensis* (L.) Kuntze; Theaceae), the regional flora includes wild species gathered for medicinal purposes including *Panax notoginseng* (Araliaceae), *Gastrodia elata* (Orchidaceae), *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz. (Melanthiaceae), and *Angelica sinensis* (Oliv.) Diels (Apiaceae) among others. Yunnan and adjacent areas are probably the centers of origin/variation of buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae), *Panax notoginseng*, *Gastrodia elata*, *Paris polyphylla* var. *yunnanensis*, and at least one of the Chinese cardamoms (*Amomum tsao-ko* Crevost & Lemarié, Zingiberaceae; Li et al., 2011). Second, Yunnan remains one of the last Chinese provinces known for extensive apiculture of the native *Apis cerana* (Tan et al., 2003; Yang, 2005).

Documentation of breeding systems and plant–pollinator interactions in economically important plants of southwestern China—Including all the pollinator-dependent food and medicinal plants of Yunnan is difficult due to the general dearth of information and regional diversity of edible-medicinal species. Consequently, to advance current understanding of pollination of edible and medicinal plants in Yunnan, we selected a representative subset of 11 species for literature review and/or pollination analysis. Buckwheat, oilseed rape (*Brassica campestris*, *B. napus*, and their hybrids; Fig. 2A), and tea oil (*Camellia oleifera* C. Abel) are reviewed here because they are of major economic importance in Yunnan and there are Chinese publications on their pollination. In fact, while Yunnan is a major Chinese center for commercial tealeaf production, tea oil trees were introduced only recently (Xie et al., 2013). We included *Gastrodia elata*, *Paris polyphylla* var. *yunnanensis*, and *Panax notoginseng* as medicinal species and dietary supplements first cultivated in Yunnan; *Dendrobium catenatum* Lindl. (Orchidaceae), also a medicinal species native to Yunnan but first cultivated elsewhere; and a native cardamom, *Amomum tsao-ko*, largely cultivated in warm lowland areas in Yunnan and used as a traditional spice and medicine (Table 1).

Finally, we include *Codonopsis subglobosa* (Campanulaceae), an endemic, perennial vine that the Naxi people in Lijiang, northwestern Yunnan use as a medical substitute for the better known *C. pilosula* Franch. Curiously, roots of *C. pilosula* are used as less-expensive substitutes for ginseng powders and tinctures. *Codonopsis subglobosa*, found commonly along forest margins in Lijiang, flowers and fruits between July and October. Because it prefers disturbed sites, the Naxi people protect and cultivate the vines that invade their vegetable gardens (Fig. 2D). All the information on the breeding and pollination system of *C. subglobosa* described next represents original research.

Breeding system and pollination of Codonopsis subglobosa—We conducted hand-pollination experiments and pollinator observations in a population in Jinshan, Lijiang (26°46′606″N, 100°17′139″E, altitude 2369 m a.s.l.) in 2011. This population of 10 flowering stems is located between a forest and a cornfield (*Zea mays* L.). Each flowering stems produced >100 flowers.

As in the majority of flowers in the Campanulaceae, the stigma functions as a secondary pollen presenter (see Bernhardt, 1996) and does not enter a female phase (stigma receptive) until the third day of its floral lifespan. To test for self incompatibility, we conducted four hand-pollination treatments in August 2011: (1) bagged control—2–3 floral buds/stem were enclosed in nylon bags during flowering but never hand-pollinated (N = 45); (2) hand self-pollination—2–3 bagged buds/stem were allowed to open in the nylon bags; when the receptive stigma emerged, it was hand-pollinated with grains from the surrounding, sterile stylar region (N = 32); (3) hand cross-pollination—mature buds were emasculated before the corolla opened, stigmas were hand-pollinated with grains from 10-m distant plants still in the male, pollen-presenting phase (N = 27); (4) open pollination—flowers were tagged but never bagged (N = 43), and mature fruits and seeds were collected and counted in October.

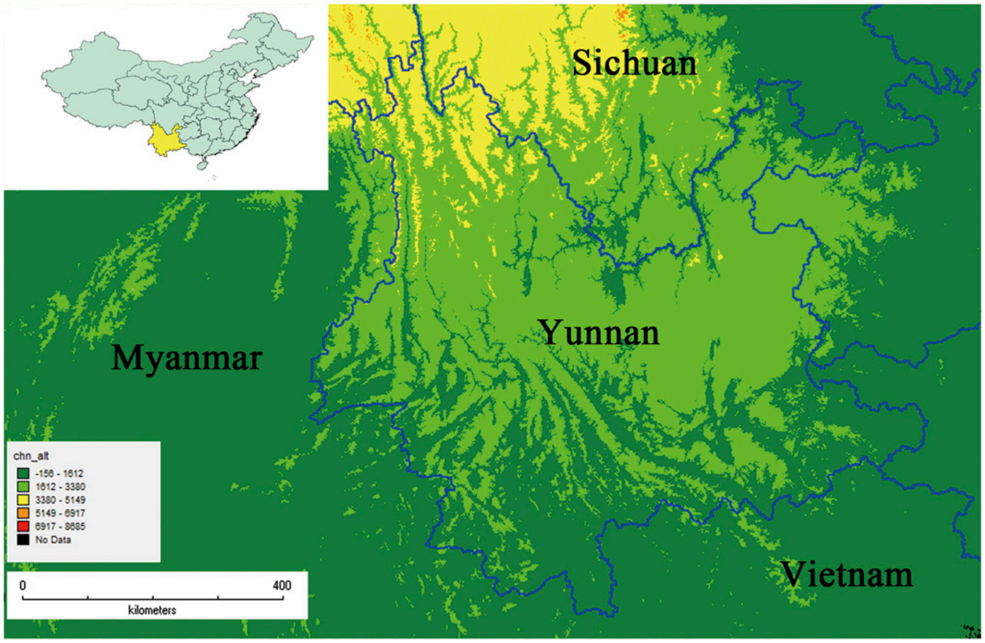

Fig. 1. Location of study area. Yunnan Province is in southwestern China, bordered by Myanmar and Vietnam. Topography of Yunnan is mountainous with elevations above 1500 m.

Seed production of different treatments was compared by one-way analysis of variance (ANOVA). If significant differences were detected, Tukey's honestly significant difference (HSD) post-hoc test was used to determine the sources of the differences.Statistical analyses were performed in R (version 3.0.2, R Development Core Team, 2013).

The flowers of *C. subglobosa* always opened in the morning and lasted about 7 d. Floral visitors were observed for 3 d from 10:00 to 18:00 hours. Because the visitation rates were high, we observed one plant with about 50 flowers for 15 min each hour (total observation time: 5.5 h). The total number of insect visitations was recorded. Foraging behavior, including visible deposits of pollen of *C. subglobosa*, was recorded and photographed. Insect visitors were caught, euthanized using fumes of diethyl ether, pinned, identified, and deposited as vouchers in the Kunming Institute of Botany, Chinese Academy of Sciences. Dr. Xin-Ming Zhang from Southwest Forestry University identified wasp specimens.

Ethnobiological survey of Apis species in northwestern Yunnan—Yunnan is one of the last refuges of the eastern Asian honeybee, *Apis cerana*, in China with wild populations found in northwestern Yunnan (Tan et al., 2003; Yang, 2005). We reviewed the literature on how this species is kept and conducted an ethnobiological survey in 2013 to understand why stocks of this honeybee decreased in Wangjiawuji, a small village in Lijiang, northwestern Yunnan. The local villagers have traditional ways of introducing wild honeybee colonies to manmade hives. We asked 10 residents who were all either beekeepers or former beekeepers the same three questions: (1) How many honeybee hives do your family and your neighbors keep today? (2) How many honeybee hives did this village have 10 years ago? (3) Do you know why people here now keep fewer hives?

RESULTS

Breeding systems of selected species and their pollinators—A review of the literature showed that *Panax notoginseng* was the only species examined here in which both the breeding system and pollinators remain unknown. Four species were self-compatible, and four were self-incompatible. Following experimentation, *Codonopsis subglobosa* showed a partial self-incompatibility response, based on seed set and was the only species pollinated by true hunting wasps (see next section). *Camellia oleifera* depended exclusively on two indigenous species of solitary bees. *Angelica sinensis* and *Fagopyrym esculentum* may depend on a broader range of generalist foraging insects. Importantly, *Gastrodia elata*, *Dendrobium catenatum*, and *Paris polyphylla* var. *yunnanens* can persist in cultivation only through hand-pollination (Table 1).

Breeding system and pollination biology of Codonopsis subglobosa—We found that *C. subglobosa* was self-compatible but dependent on pollinators to maximize seed set. Mechanical self-pollination (autogamy) occurs with some frequency as 47% of the bagged control flowers develop into fruits (Table 2). However, seed production was significantly lower in bagged controls (272.33 ± 27.87, N = 21) compared with open-pollinated flowers (949.19 ± 45.63, N = 36; Tukey's HSD test, P < 0.001). Fruit set of hand self- and cross-pollinated flowers was 87.5% and 92.59%, respectively, but no significant difference statistically (χ^2 = 0.0451, df =1, P = 0.8317). Hand-self-pollinated fruits produced significantly fewer developed seeds (one-way ANOVA: $F_{3, 106}$ = 72.97, P < 0.001). Ovaries of open-pollinated flowers showed an 84% conversion ratio into fruits.

During the 5.5 h observation period, we recorded the predatory wasp (*Vespa velutina auraria* Smith) and the native *A. cerana* visiting the flowers. Female wasps were the dominant foragers (Fig. 2I). We observed them entering flowers 641 times. They hung upside down from the corolla lobes before entering the flowers. A wasp usually climbed up toward the base of the style to consume copious droplets of nectar. Visible quantities of pollen of *C. subglobosa* adhered dorsally to their heads, thoraces, and abdomens as they contacted the pollen presenter on their way to the basal nectar glands (Fig. 2I). In contrast, we only recorded 17 workers of *A. cerana* visiting the flowers. When we discussed this species with local farmers, they told us they preferred to avoid these plants in bloom for fear of the wasps.

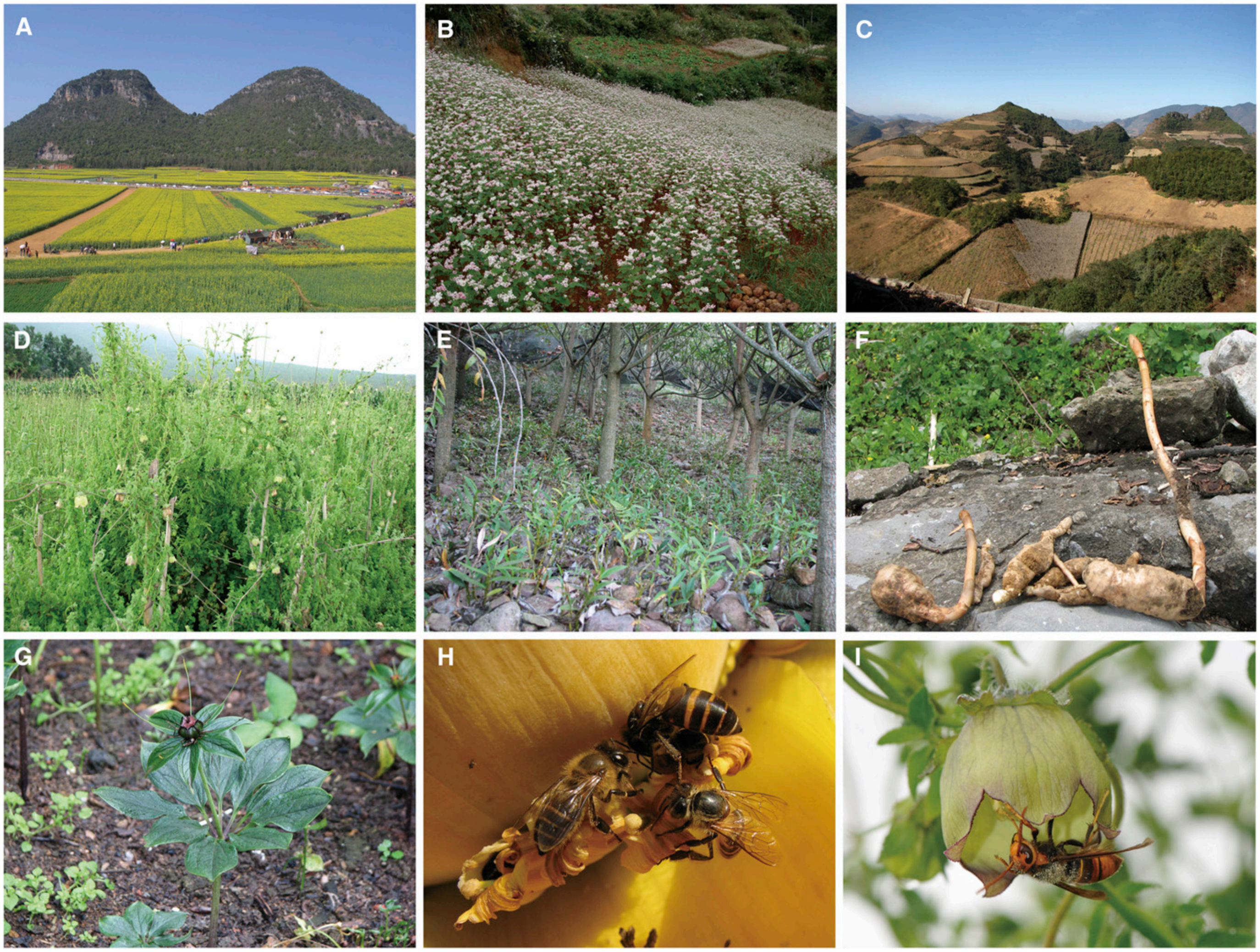

Fig. 2. Crops and their pollinators in Yunnan, China. (A) Rapeseed crop in bloom with Karst mountains on the horizon. Note the hives of *Apis mellifera* in the field. The beekeeper provides free pollination services for all bee-pollinated crops in Yunnan (Photo by Mr. Hua-Jie He). (B) Buckwheat (*Fagopyrum esculentum*) field in northeastern Yunnan. (C) Plantation of *Panax notoginseng* in Wenshan, southern Yunnan. Due to replant disease (see *Available information on breeding systems and pollinators of edible/medicinal species in Yunnan*), farmers need to clear more native forest to plant a second crop, leading to forest fragmentation in these areas. (D) Example of companion planting in Lijiang, northwestern Yunnan in which medicinal species are grown as a cash crop among *Zea mays*, which corn will be harvested for livestock feed. Note also that *Codonopsis subglobusa* and *Dipsacus asperoides* (Dipsacaceae) are grown together. (E) Plantation of *Dendrobium cantenatum* in Jingdong County, Yunnan. Seedlings are grown in vitro, then sold to farmers to grow outdoors. (F) Illegal collection of wild specimens of *Gastrodia elata* in northeastern Yunnan. (G) Fruiting plant of *Paris polyphylla* var. *yunnanensis*. (H) Workers of *Apis cerana* visit male flowers of a *Musella lasiocarpa* (Musaceae). (I) Female of *Vespa velutina* visits *Condonopsis subglobosa.* Note the dorsal deposition of pollen on the thorax (Photo by Dr. Zhi-Kun Wu).

Evidence for the decline of Apis cerana apiculture in Yunnan—A review of the literature indicated that wild populations of *A. cerana* were harmed by the introduction of Italian strains of *A. mellifera* due to a combination of interspecific competition and disease (Yang, 2005). The natural distribution area of *A. cerana* has now been reduced by 75% in China, while the number of traditional hives maintained has decreased by over 80% (Yang, 2005). Currently, large wild populations of *A. cerana* are found almost exclusively in the southwestern mountain ranges of China, especially in northwestern Yunnan. Compared with *A. mellifera*, *A. cerana* has several traits that are important to traditional Chinese agriculture. First, *A. cerana* continues to forage and pollinate small populations of native montane species important as wild foods and medicines. Workers in the same hives also forage on crops that flower sparsely in diverse but small village gardens. *Apis cerana* starts foraging earlier in the season, and hive population numbers peak earlier than *A. mellifera* (Tan et al., 2012). Second, *A. cerana* is better adapted to more diffuse nectar resources than *A. mellifera* (Yang et al., 2013). Third, *A. cerana* evolved with native *Vespa* and other hunting wasp species, and these bees have special antiwasp defenses such as shimmering. Workers also form a "killing ball" around the wasp to fatally increase its body temperature (Tan et al., 2013).

The number of hives of *A. cerana* also declined in villages still practicing traditional apiculture in northwestern Yunnan. Our 2013 interviews with the remaining beekeepers in Wangjiawuji indicated that, before the year 2000, each apiculturist kept 2–12 hives of *A. cerana.* Collectively, these beekeepers kept about 49–53 hives. Five of these people no longer kept hives.

Table 1. Breeding systems, pollinator, usage, and reproduction of medicinal/food plants in southwestern China.

Species	Family	Breeding system	Pollinator	Uses	Propagation	References
Angelica sinensis	Apiaceae	C	Wild insects	M/D	Seed	Liang et al., 2010
Amomum tsao-ko	Zingiberaceae	C, flexible style	Honey bees	S	Seed/rhizome	Cui et al., 1995
Brassica napus and *B. campestris*	Brassicaceae	C	Honey bees, wild insects	O	Seed	Klein et al., 2007
Camellia oleifera	Theaceae	SI	Native solitary bees	O	Seed/cutting	Deng et al., 2010
Codonopsis subglobosa	Campanulaceae	PSI	Predatory wasps	M/D/V	Seed	This study
Dendrobium catenatum	Orchidaceae	SI	Hand pollination	M/D	Seed	Li et al., 2009
Fagopyrum esculentum	Polygonaceae	SI	Honeybees, wild bees, hover flies	E/M	Seed	Cawoy et al., 2008
Gastrodiaelata	Orchidaceae	C	Hand pollination	M/D	Seed	Zhou et al., 2005
Paris polyphylla var. *yunnanensis*	Melanthiaceae	SI	Hand pollination	M	Seed/rhizome	Yang et al., 2008
Panax notoginseng	Araliaceae	unknown	Unknown	M/D	Seed/rhizome	

Notes: Breeding systems—C = self-compatible but requires pollen vector, PSI = partially self-incompatible, SI = self-incompatible. Uses—D = dietary supplement/food additive, E = edible carbohydrate, seed, M = medicinal, O = oil, S = spice, V = vegetable. Pollinators listed are in cultivated environments. Hand pollination refers to flowers pollinated by human hand when natural pollination is insufficient or lost.

Local people can still find wild colonies of this bee in the forest and take them home. They render the colony docile using a gentle smoker containing smoldering, dried stems of *Leontopodium* species (Asteraceae). Colonies are kept as a honey source not as a crop pollinator resource. After 2000, this number of wild-collected colonies decreased to 10–15 hives in the entire village. The 10 local/former beekeepers interviewed for this study gave four reasons for a decline in their interest in collecting and maintaining wild colonies (Table 3). First, widely used pesticides killed *A. cerana.* Second, the Chinese celebrate their Spring Festival (January–February) using fireworks. They believe that the noise and smoke aggravated honeybees in artificial hives located around their homes and yards. Third, some villagers insisted that hornets "scared away" the new queens, causing the colony to swarm and vacate. Traditional beekeepers also believed they needed to repel native hornets, especially on hot summer days, by waving tree branches at the predators as they flew toward the hive, a seasonally tiresome exercise (Z.-X. Ren, unpublished data, personal experience).

Their fourth reason suggested a decline in interest due to selective deforestation by fellow villagers. During our interviews, former traditional beekeepers complained that honey volume had decreased in their hives over two decades. This decline may have been the result of local forestry policies. The wooded areas around the village and the city of Lijiang are dominated by pine (*Pinus yunnanensis* Franch.; Pinaceae) and several *Quercus* species (Fagaceae). These trees, of course, do not offer floral nectar. Local villagers are either not allowed or need permission from the Forest Department to log pine trees for construction or cut oaks for firewood. Thus, the villagers instead cut *Docynia delavayi* (Franch.) C.K. Schneider (Rosaceae), *Prunus mume* Siebold & Zucc. (Rosaceae), and other woody species, which are not regulated because they are less desirable for wood. However, these woody species once provided seasonal nectar flows, according to five of the people interviewed, and they noticed the loss of trees (Table 3).

Table 2. Fruit set and seed production of pollination experiments of *Codonopsis subglobosa*.

Treatments	No. flowers	No. fruits	Fruit set (%)	Seed production (mean ± SE)
Self-pollination	32	28	87.50	772.07 ± 33.98
Cross-pollination	27	25	92.59	1353.60 ± 69.27
Open-pollination	43	36	83.72	949.19 ± 45.63
Bagged control	45	21	46.67	272.33 ± 27.87

DISCUSSION

Available information on breeding systems and pollinators of edible/medicinal species in Yunnan—It is important to note that self-incompatibility persists in both recently cultivated, semidomesticated species (*Dendrobium catenatum* and *Paris polyphylla* var. *yunnanensis*) and in cultivars or landraces of *Camellia oleifera* and *Fagopyrum esculentum* (Table 1). The persistence of self-incompatibility in these crops suggests that, as in other civilizations over time, both traditional and modern agricultural practices in China could not always overcome ancestral self-incompatibility mechanisms to increase crop yield by a process of selective breeding. While six species (*Amomum tsao-ko*, *Angelica sinensis*, *Codonopsis subglobosa*, *Gastrodia elata*, and some recent cultivars of *Brassica campestris* and *B. napus*) have some degree of self-compatibility, these species, or their cultivars, continue to require insect-mediated pollination to maximize seed set (Table 1). This requirement is not unique to edible and medicinal crops in China. We remind readers that, Darwin (1876) was among the first to experiment on the breeding systems of a number of domesticated species. He noted that many required vector-mediated cross-pollination to maximize fruit and seed set even though they would set some fruit and viable seeds when self-pollinated by hand.

Amomum tsao-ko—This member of the Zingiberaceae is a highly priced spice and traditional medicine, cultivated for its multiseeded fruits. Both wild and cultivated populations are found only in Yunnan where it borders other provinces. Cui et al. (1995) found that the flowers were heterodichogamous. Li et al. (2001) found two flexistylous floral forms on the same plant. One morph is anaflexistylous (protogynous), while the second is cataflexistylous (protandrous). This system promotes self-isolation within each flower because both forms open synchronously on the same stem. A plant can be self-pollinated by hand if pollen is transferred from the cataflexistylous flower to the anaflexistylous flower (Cui et al., 1995; Li et al., 2001). Both *Apis cerana* and *A. mellifera* pollinate cultivated plants (Li et al., 2011).

Angelica sinensis—In contrast, *A. sinensis* remains a common herb of vegetable gardens in northwestern Yunnan with a

TABLE 3. Factors the local beekeepers attributed to the decline of native honeybee hives in Wangjiawuji, northwestern Yunnan (yes = 1, no = 0).

Beekeeper	Pesticides	Fireworks	Predatory wasp attack	Deforestation of nectar resources	Other (unknown reason)
1	1	1	0	0	0
2	1	1	0	1	0
3	1	1	0	1	1
4	1	0	1	1	0
5	1	1	1	0	0
6	0	0	1	0	0
7	1	0	0	1	0
8	1	1	0	0	0
9	1	0	1	0	0
10	1	1	1	1	0
Ratio	0.9	0.6	0.5	0.5	

natural distribution in Yunnan and provinces through the Himalayas. The root of *A. sinensis* is widely used as a medicine and diet supplement for gynecological ailments, fatigue, mild anemia, and high blood pressure. It is self-compatible, but insects may be needed to increase fruit and seed set (Liang et al., 2010). Traditionally, local farmers propagated them from seeds.

Brassica napus and B. campestris—Oil seeds are extremely important in Chinese agriculture for edible and industrial purposes. Rapeseed (*Brassica napus* and *B. campestris*) production in China accounts for 30% of total production worldwide. However, China's huge consumption of cooking oils makes the country one of the largest importers of raw rapeseed and processed oil (FAOSTAT, 2014). *Brassica campestris* and *B. napus*, two of the most important economic crops in Yunnan were derived from species with typical (*Brassica*-type) early-acting self-incompatibility (Takayama and Isogai, 2005), but cultigens grown in Yunnan today are self-compatible. Insect pollination is known to increase the yield in these self-compatible cultigens (Free, 1970; Klein et al., 2007).

A controlled experiment using European strains of *A. mellifera* and native *A. cerana* to pollinate *B. campestris* in Yunnan showed that when *A. mellifera* pollinated flowers in open fields, seed production increased by 32.78%. In control cages, *A. mellifera* increased yield only 3.02%, while *A. cerana* increased seed production 16.89% (Zhou et al., 2010). These results may indicate that *A. cerana* is a more efficient pollinator when floral resources are sparse and discrete. In the absence of bees, modern cultigens of rapeseed self-pollinate mechanically, fewer fruits and smaller seeds are produced compared with those produced after insect pollination (Khan, 1995).

Camellia oleifera—*Camellia oleifera* is pollinated by non-*Apis* bees (Deng et al., 2010; Xie et al., 2013). Because the floral nectar appears to be so toxic to *Apis cerana* and *A. mellifera*, colony numbers of both bee species decline when their hives are placed in or near *C. oleifera* plantations. Nectar analyses tested positively for caffeine, raffinose, stachyose, and galactose, which kills workers of both *Apis* species (Su et al., 2012). The native pollinators of *C. oleifera* are *Andrena camellia* (Andrenidae) and *Colletes gigas* (Colletidae; Wu, 1977; Ding et al., [AQ1] 2007, 2010). Both species forage for nectar without evidence of toxic symptoms. As tea oil consumption increased over the past decade, so did the planting area of *C. oleifera* in southern China. However, fruit production remained low in newly cultivated areas due to the regional absence of *Andrena camellia* (Xie et al., 2013). It is relevant to note that, to maximize fruit and seed set, shrubs of *C. oleifera* retain their specialized pollinators through domestication as in the more famous case of shrubs of *Theobroma cacao* (Young, 1994).

Dendrobium catenatum—This orchid is one of at least 40 *Dendrobium* species used in traditional Chinese medicine (Bulpitt et al., 2007). Self-incompatibility occurs extensively within this genus of more than 1100 species (Johansen, 1990), and cultivated species in China showed extremely low fruit set following self-pollination by hand (Li et al., 2009). *Dendrobium catenatum* is one of the highly priced medicinal and dietary supplements in China (Liu et al., 2011b). Modern mass propagation of plants depends exclusively on rapid seed culture in vitro. Throughout China, the *D. catenatum* industry involves companies providing industrial laboratories with aseptic culture while working with local farmers (Fig. 2E; Liu et al., 2013). The industry is dependent entirely on hand-mediated cross-pollination (Liu et al., 2011b). The over-collection of wild plants led to the extirpation of local populations in China and southern Asia (Liu et al., 2011b, 2013). Although 78 *Dendrobium* species are distributed through China (Liu et al., 2011b), the pollination ecology of the majority of species, including *D. catenatum*, remains unknown.

Dendrobium sinense Tang & F.T. Wang is pollinated exclusively by females of the predatory wasp, *Vespa bicolor* (Brodmann et al., 2009). The flowers do not offer edible rewards, and the wasps mistake the floral odor of the orchid for the warning pheromone of its prey, *Apis cerana*. Food-mimic orchids, like *Dendrobium* spp., tend to have lower conversion rates of flowers into fruits (Tremblay et al., 2005). However, we note that the commercial *Vanilla* industry has thrived in Mexico and in other tropical countries since the second half of the 19th century using hand-pollination techniques (Cameron, 2012).

Fagopyrum—Historically, buckwheat has attracted traditional consumers and herbalists as a food source and for its use in treating chronic diseases (He et al., 1995; Zhang et al., 2012). The Yi ethnic minority people in Sichuan and Yunnan cultivate common buckwheat (Table 1) at low elevations and tartary buckwheat [*F. tataricum* (L.) Gaertn.] at higher elevation as their staple carbohydrate (Fig. 2B). Tartary buckwheat is a homostylous and autogamous species (Fesenko, 2005). There are no reports, at present, of insect pollinators visiting this crop.

In contrast, common buckwheat is distylous and self-incompatible (Cawoy et al., 2008). Yunnan and neighboring southern Sichuan are the center of origin and variation of both *F. esculentum* and *F. tataricum*, and wild relatives of buckwheat grow in these areas (Ohnishi and Matsuoka, 1996; Ohnishi, 1998). The local Yi minority still cultivates some landraces based on wild populations although wild *Fagopyrum* species are endangered. Worldwide cultivation of *F. esculentum* depends primarily on pollination by commercial *Apis mellifera*, but these insects are often regarded as inefficient based on low yields in North America (Björkman and Pearson, 1995). In contrast, one case study in northern China suggested that generalist pollination featuring a broader range of native bee species (25%), hover flies (Syrphidae, 27%), and Lepidoptera (13%) increased yield. Honeybees (*A. cerana* and *A. mellifera*) accounted for the additional 35% of recorded visitations (Wang and Li, 1998).

A case study of *A. cerana* pollination of common buckwheat found that this bee began its foraging activities early in the morning and ceased late in the evening, a period of approximately 10 h/day. Pollination by *A. cerana* increased seed mass and the conversion of ovaries into fruits, increasing yield and fruit size substantially (Singh, 2009). Although China is one of the biggest buckwheat producers internationally, the national buckwheat crop of China decreased from 2.5 million ha in 1961 to 0.74 million in 2011 (Fig. 3; FAOSTAT, 2014). From 1974 to 2004, the mean yield in China was 1445.9 kg/ha. After 2005, the mean yield dropped to 802.8 kg/ha (FAOSTAT, 2014). There are no published explanations for this decline.

Gastrodia elata—Unlike *Dendrobium catenatum* (see above), *G. elata* is an aphyllous and obligately mycotrophic member of the Orchidaceae distributed through temperate, deciduous forests of eastern Asia. It depends on the mycorrhizal fungus *Armillaria mellea*, a key wood decomposer, for its nutrients (Zhou et al., 1987; Cha and Igarashi, 1995). The root tubers of *G. elata* are used as Chinese traditional medicine for treating headaches, dizziness, tetanus, and epilepsy, indicating neural protective functions and as a dried additive in local meat dishes (Tsai et al., 2011). As described already, the tubers of *G. elata* were once wild collected but over-exploitation of reduced native Chinese populations of *G. elata* is leading to local extirpation (Fig. 2F; China Plant Specialist Group, 2013). *Gastrodia elata* has been grown as a crop since the 1970s. In northeastern Yunnan, one of the largest production areas, they are grown in woody composts under shade screens (Zhou et al., 1987). Like most orchids, *G. elata* is self-compatible, but it is not capable of mechanical self-pollination and must be hand-pollinated for seeds to produce the next generation (Zhou et al., 2005). In

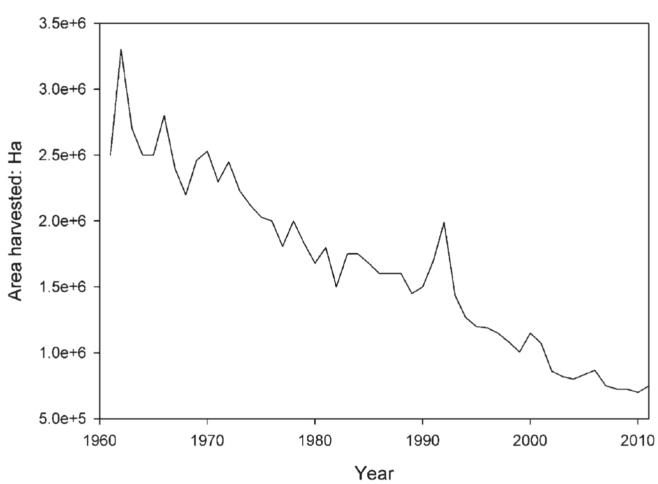

Fig. 3. Decline in harvest area of buckwheat in China from 1961 to 2011 (data provided by FAOSTAT, 2014).

China, vegetative propagation techniques are still used by subdividing and replanting tuber segments. However, all large crops of this orchid start with seeds. In Japan, the flowers of *G. elata* do not secrete nectar; female bees of a *Lasioglossum* sp. (Halictidae) are the primary pollinators. The bees collect epidermal cells shed by the labellum (Kato et al., 2006). The same mode of visitation and reward was observed by Jones (1985) for *G. sesamoides* in Victoria, Australia, and females of *Exoneura* sp. (Apidae) were the primary pollinators.

Panax notoginseng—Like the renowned root of *Panax ginseng* used as a traditional tonic against fatigue and other ailments for over 2000 yr (Wen and Nowicke, 1999), the root of the lesser-known *P. notoginseng* is one of the most highly valued Chinese medicinal herbs. *Panax notoginseng* has been cultivated for more than 400 yr in southeastern Yunnan, and it remains the most important notoginseng-producing area in China (Zheng and Yang, 1994). Due to a long history of domestication and over-collection of wild populations, *P. notoginseng* is believed to be extinct in the wild in China (Wang et al., 2008). The traditional propagation of notoginseng is dependent on both root tuber division and seeds. However, due to the loss of genetic diversity in domestication, cross-pollination by hand for seed production is considered very important to maintain genetic resources because notoginseng suffers from "replant disease". Specifically, when a second crop is planted in the same site as the previously harvested crop, root production of the second replacement crop declines dramatically (Zhang and Lin, 2009). To avoid this problem, notoginseng farmers continuously clear new sections of native forest to cultivate more notoginseng. This practice fragments forests in Wenshan County, which is the main area of notoginseng cultivation in Yunnan (Fig. 2C).

We still don't know whether this species has a self-incompatibility mechanism, and the pollinator(s) remains unknown. The North American ginseng, *P. quinquefolius,* is known to be self-compatible, but plants grown from self-pollinated seeds are smaller and appear to grow more slowly than seeds produced by cross-pollination (Mooney and McGraw, 2007). Pollen vectors of the North American species include a surprisingly wide variety of arthropods, but the primary source of pollen transfer between plants are woodland bees in the genus *Dialictus* (Halictidae; Catling and Spicer, 1995).

Paris polyphylla var. yunnanensis—In Yunnan, the tuberous roots of *P. polyphylla* var. *yunnanensis* are ingested traditionally to treat bleeding and as an "immunity adjustment" and analgesic (Fig. 2G; He et al., 2006). All resources were derived originally from wild populations with over-exploitation leading to local extirpations. This perennial herb must also be cross-pollinated by hand to produce the next generation of seeds. When hand self-pollinated, only 21% of ovaries matured into fruits, and they contained fewer seeds than in fruits that developed after cross-pollination (Yang et al., 2008). The breeding system of *P. polyphylla* var. *yunnanensis* has never been analyzed using standard epifluorescence techniques, so we do not know whether this species has an early- (sporophytic) or late-acting (gametophytic) self-incompatibilty system. Some species of *Trillium*, the sister genus of *Paris* in the family Melanthiaceae, however, are known to have early-acting self-incompability (Sage et al., 2001). Li (1998) suspected that woodland flies pollinated *P. polyphylla* var. *yunnanensis* but had no field observations as evidence. It is surprising that hand-pollination of *Paris polyphylla* var. *yunnanensis* dominates propagation because resident guilds of woodland pollinators should remain since these plants are now grown commercially in modified forests. The shortage of pollinator-mediated pollination under cultivated environments suggests a deficit of specialized pollinators.

The specialized pollination biology of Codonopsis subglobosa and its implications for Yunnan flora and crop evolution—*Codonopsis subglobosa* is cultivated but not domesticated and remains a regional/ethnic crop (see above). As in some of the species Darwin (1876) studied, our experiments showed that *C. subglobosa* is only partially self-incompatible. It can self-pollinate mechanically, but cross-pollination greatly enhances seed set, and wasp-mediated pollination produces more seeds than the act of mechanical autogamy. The plant also appears to depend on a relatively narrow spectrum of pollinators, as does *Camellia oleifera* (see above), but additional research on *C. subglobosa* and other native/cultivated species in Yunnan is needed to confirm this perception. While the vast majority of angiosperm species are not pollinated primarily by hunting wasps (see review by Bernhardt et al., 2013), the pollination ecology of *C. subglobosa* and *Dendrobium sinense* (Brodmann et al., 2009) may signify that the evolution of hunting wasp pollination systems has evolved repeatedly in unrelated species native to southwestern China. Perhaps this region of China is a center for the convergent evolution of hunting wasp pollination similar to southern Africa, a center for the convergent evolution of pollination systems dependent on anthophilous scarabs known as "monkey beetles" (see Bernhardt, 2000). With so many angiosperm species in China cultivated, but not actually domesticated, there are probably many more edible and medicinal species that are pollinated exclusively by one or a few species of pollen vectors such as wasps, or bees, or birds, etc. As in our brief study of *C. subglobosa*, simple field techniques and protocols combined with the cooperation of traditional farmers and horticulturists can clarify at least part of the life history of a regionally important crop. Of greater importance, China could become a center for the study of the reproductive biology of cultivated species in early or transitional periods of domestication.

Comparative roles of A. cerana and A. mellifera in the Yunnan landscape—*Apis mellifera* is Eurasian and African in distribution, but the center of diversity for the genus is in subtropical-tropical Asia and its archipelagos (Michener, 2000; Smith et al., 2000; Oldroyd and Wongsiri, 2006). The two *Apis* species now kept by Chinese beekeepers are cavity nesters (Oldroyd and Wongsiri, 2006). China has a much older history of domestication of *A. cerana* compared with the commercial Eurasian honeybee. *Apis mellifera* was introduced into China by Europeans during the second half of the 19th century (Kevan, 1995; Yang, 2005). Five native *Apis* species distributed in China (*A. cerana*, *A. florea*, *A. dorsata*, *A. andreniformis*, and *A. laboriosa*), approximately half of the genus, are native to Yunnan. Wild and wild-collected populations of *A. cerana* are widely distributed throughout the various geographic regions and climate zones of Yunnan. It is the only semidomesticated *Apis* species in China (Fig. 2; and see Tan et al., 2003). Nevertheless, beekeeping in China shows a rampant decline in the use of this native, wild-collected species, even in Yunnan, compared with the expanding use of *A. mellifera*. The primary reason is that Chinese agronomists consider *A. mellifera* to be more efficient at increasing yields of various crops when such crops are grown in intensive, extensive monocultures (Westphal et al., 2003).

While the rental market for honeybee hives (*Apis mellifera*) for pollination services is well developed, lucrative, and organized in North America (Sumner and Boriss, 2006) and Europe (Breeze et al., 2011), beekeepers of *A. cerana* and A. *mellifera* in China continue to provide free services for crops. Therefore, the specific income for beekeeping in China is based exclusively on honey and wax production. As usual Chinese apiculturists must move their hives to exploit seasonal crops blooming on a brief mass scale (Fig. 2A), but farmers do not invite beekeepers into fields in Yunnan.

The economic value of honeybee pollination for 36 crops in China from 2006 to 2008 was estimated at 304.22 billion yuan (about US$50.2 billion), equivalent to 76 times the value of honey and wax, constituting 12.30% of the gross output value of Chinese agriculture. There was a great demand for honeybee pollination in vegetables, fruits, cotton, and other crops in 2008, and an estimated 60–88 million colonies needed to be deployed throughout China (Liu et al., 2011a). However, these estimates were all based on publications from other countries. Original data on how *A. cerana* and *A. mellifera* can increase yield in a far wider variety of Chinese crops in different locations is lacking.

Other questions left largely unanswered are the impacts of the relatively recent introduction of *A. mellifera* on the pollination of the native flora of China, on remaining wild populations of *A. cerana* and on agricultural and horticultural practices in rural (particularly mountainous) areas. Agricultural, apicultural, and horticultural practices using native *A. cerana* have been employed for thousands of years compared with the recent use of *A. mellifera* (Yang, 2005; You et al., 2005).

The advance of the culture of *A. mellifera* in China does not automatically guarantee an increase in the yields of bee-pollinated crops because of the impact of other current and prevailing agricultural practices. As a typical example highly cited by international media, farmers in several counties in Sichuan Province have to pollinate apple and pear flowers manually using "pollination sticks" that are nothing more than wooden brushes tipped with chicken feathers or cigarette filters (Tang et al., 2003; Partap and Tang, 2012). Because they are clonally propagated, apple trees of a single cultigen are both self-incompatible and self-intraincompatible, hand pollination cannot transfer sufficient pollen from donor trees (belonging to other intercompatible cultigens), nor is it possible to maximize fruit yield using hand pollination. Furthermore, beekeepers simply refuse to bring their hives into these orchards due to the indiscriminate use of pesticides (Tang et al., 2003) (Table 3). We presume that this pesticide use also kills resident populations of native pollinators, which may be particularly devastating to pear crops. Free (1970) noted that *A. mellifera* does not forage preferentially on pear flowers, and pear pollination rates may increase due to small native bees and hoverflies (Syprhidae). When we compared the yield of apples and pears between China and the United States, apple yield in China is 11.10–54.88% lower, and pear yield was 14.07–42.81% lower (Fig. 4). These yields may, in part, indicate an urgent need for new pollination management policies in China.

Past and future of insect-pollinated and traditional crops in southwestern China—China has a long history of ethnobotanical research and ethnography (Hu, 2005), but pollination biology must be regarded as a younger subdiscipline. In Europe, pollination biology emerged as a discrete and synthetic discipline within botanical research by the second half of the 19th century (Proctor et al., 1996). This emergence, in part, was due to Charles Darwin

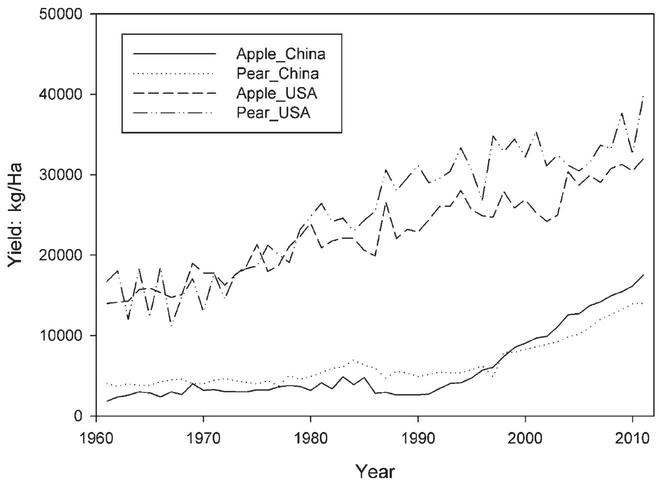

Fig. 4. Comparison of apple and pear yield in China and the USA (data provided by FAOSTAT, 2014)

realizing that pollination is intrinsic to the study of natural selection (Allan, 1977) and extending his observations to edible crops and ornamental plants. In China, consistent, long-term field studies in this discipline are less than 20 yr old (Huang, 2012). In a recent review on pollination ecology in China, most publications were found to focus on wild species (Fang et al., 2012). Pollination ecologists and entomologists in China should broaden their research areas to both ethnic and industrial agroecosystems (Mitchell et al., 2009). The ethnic and rural systems are of particular importance due to the sheer diversity of edible, fiber, and medicinal species under cultivation (López-Pujol et al., 2006; Li and Pritchard, 2009; Huang, 2011).

Hand pollination of certain crops will continue in China, and the practice will probably expand until agricultural protocols change. What is needed now, though, are new hand-pollination techniques that avoid low yields and decrease current trends in inbreeding depression to produce next season's seeds for planting. We suggest that Chinese laboratories devote more space, time, and equipment to locating self-recognition sites of pollen tube rejection in the pistils of preferred landraces and wild species undergoing a long domestication process. We suggest investment in microscopy techniques designed to follow the progress and viability of sperm in individual pollen grains and in pollen tubes growing through carpels. We also argue that identification of key pollinators is incomplete unless microscopy is used to determine whether the floral forager is carrying significant quantities of pollen on portions of its body where such grains are most likely to contact receptive stigmas. In addition, a wider variety of staining techniques should be used to determine when stigmas are viable, how many pollen grains germinate on stigmatic surfaces, and how many pollen tubes enter ovaries and penetrate ovules (Dafni et al., 2005). It is worth time and resources to conduct controlled experiments to determine the number of viable pollen grains deposited on receptive stigmas that result after recorded visits by different pollen vectors (Kearns and Inouye, 1994).

To fulfill the demands of food consumption, China will probably continue to use western methods of agriculture, devoting vast areas of land to monocultures (Godfray et al., 2010). Pollination services, as in the West, will have to be added to the cost of production. The same techniques will probably apply to plant species preferred as medicines and dietary supplements as native populations dwindle. The applied science of pollinator management awaits Chinese investment and invites international collaboration.

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