

Traditional home-garden conserving genetic diversity: a case study of *Acacia pennata* in southwest China

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Abstract Conserving biodiversity in human-dominated systems requires research into mechanisms that can maintain biodiversity in fragmented landscapes. Home-garden as traditional agroforestry system in many regions has shown great value in maintaining a wide range of species. Here we show that home-garden populations are also capable of maintaining high level of genetic variation. Using six polymorphic microsatellite DNA markers, we have genotyped 260 individuals of *Acacia pennata*, a popular wild vegetable in the tropical region of southeast Asia. Samples were collected from home-gardens and wild populations in Xishuangbanna, southwest China. Microsatellite DNA diversity in planted populations were compared with that in geographically nearby wild populations

with similar population size. Over 90 % of microsatellite genetic variation in wild populations was also present in planted populations. Pairwise comparison of planted and adjacent wild population showed no significant difference in allelic diversity and heterozygosity. Analysis revealed no significant genetic differences between wild and planted populations, while four home-garden populations showed sign of bottleneck. We conclude that home-gardens show great promise in maintaining genetic diversity, and that these managed patches could be of significant conservation value in tropical regions.

Keywords *Acacia pennata* · Agroforestry · Ex situ conservation · Genetic variation · Microsatellite DNA · Xishuangbanna

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Introduction

The conservation of biodiversity in agroforestry systems is vital given that human-dominated ecosystem is now a major feature of the earth's landscape (Kareiva et al. 2007; Bhagwat et al. 2008). Competent management in these areas is therefore of paramount importance (Kabir and Webb 2008; Webb and Kabir 2009). Home-garden is a traditional agroforestry system which is cultivated with a mixture of annuals, perennials and trees, and is a common feature in the majority of tropical countries. Home-gardens have recently been recognized for their potential for biodiversity conservation (Raheem et al. 2008; Kabir and Webb 2008), and for their social and cultural significance (Buchmann 2009; Rowe 2009). In many tropical regions, home-gardens have maintained diverse floristic communities with remarkable variability in composition and structure (reviewed in Webb and Kabir 2009), and are endowed

with an important ecosystem function (Hylander and Nemoissa 2008). Home-garden is the product of local peoples' acquired knowledge of domesticating useful plant species, including selecting the best traits, and propagating plants by means of seed germination or vegetative methods. With forest areas being lost to deforestation and expansive agriculture, home-garden could serve as significant repositories of germplasm (e.g. ex situ conservation).

The conservation value and long-term viability of home-garden, however, would increase if it contains a wide genetic base providing the capacity to adapt to environmental fluctuations or changes of gardening practices (Hollingsworth et al. 2005). Increasing attentions have been focused on the potential of home-garden to harbor genetic diversity, which is a key component of conservation efforts associated with population management (e.g. Hollingsworth et al. 2005; Lengkeek et al. 2006; Miller and Schaal 2006). The level of genetic diversity possessed by populations might influence their sustainability to the changes of environment, especially in small population (Ellstrand and Elam 1993; Frankham et al. 2002), which is typically faced in home-gardening practice. In home-garden populations, individuals are likely to be anatomically and physiologically similar because of the potentially artificial selection for certain traits. Whether these populations are capable to maintain and develop sufficient genetic diversity that contributes to long-term conservation value would help to evaluate the viable conservation alternative for home-garden to other forms of agroforestry.

Xishuangbanna Dai Autonomous Prefecture is a tropical area of southwestern China that borders with Laos and Burma which is floristic rich with over 4,000 vascular plant species identified in an area of 19,000 km² (Zou 1988). This region is also characterized in cultural diversity with 13 ethnic minorities. In Xishuangbanna, Dai people have tended home-garden for over 1,000 years, they collect a wide range of plants for the purpose of food, medicine, culture and religion from the area surrounding their villages, and then transplant them in their home-gardens for later use (Long and Li 2006). *Acacia pennata* (Linn.) Willd (Fabaceae) is widely distributed in tropical and subtropical southeast Asia. This diploid species is a vine-like climbing tree, wind pollinated, and with seeds usually dispersed by wind, occasionally by birds (Graham et al. 2003). Feathery leaf shoots of *A. pennata* are consumed as a popular wild vegetable in Xishuangbanna, adjacent Thailand and Laos (Wang and Long 1995; Johnson 2002). In India, *A. pennata* is regarded as a medicinal plant and is thought to have a wide range of medicinal functions (Khare 2007). This species is commonly grown in home-garden in Xishuangbanna, partially because the declining of natural populations makes collection from the wild increasingly difficult, and also because of its increasing popularity

(Johnson 2002). While the wild populations of *A. pennata* declined rapidly from the 1990s because of the rubber trees plantation in the region.

In this study, we take *A. pennata* as a case study to explore the value of conserving genetic variation of home-garden. Using polymorphic microsatellite DNA markers, our objectives are: (1) quantify the amount of genetic variation in wild and home-garden populations of *A. pennata* (2) compare the structure and differentiation of genetic variation in wild and home-garden populations (3) evaluate the effectiveness of home gardening systems in the maintenance of genetic resources.

Methods

Leaves of *A. pennata* were collected from home-gardens and wild populations at seven sites across Xishuangbanna (Fig. 1a and Table 1). At each site, attention was taken to ensure that the sample sizes of the two categories were similar so as to avoid effects of population size on genetic diversity (Leberg 2002). In home-garden, only those trees that were originally collected from wild populations, confirmed by the home-garden owner or local informants were sampled. The wild populations were also identified as possible source of material that the villagers would have collected for their home-gardens. Leaves from up to 20 plants were collected from each population.

Total genomic DNA was extracted from each sample using a modified CTAB procedure (Gao and Li 2008a). We used three samples (two from home-garden and one from wild population) screening SSR loci from a total of 82 published primers for *Acacia*. Six loci (Table 2) were chosen on the reliable and polymorphic production of interpretable microsatellite DNA profiles in a test panel. Polymerase chain reaction (PCR) amplification was performed in a total volume of 25 µl, reaction mix contained 5–50 ng of template DNA, 0.2 µmol/l of each primer, 200 µmol/l of each deoxyribonucleotide triphosphate, 10 mmol/l Tris–Cl (pH 8.3), 50 mmol/l KCl, 25 mmol/l MgCl₂ and 0.5 U Ex Taq DNA polymerase (TaKaRa, Shiga, Japan), using the following PCR profile: 94 °C for 2 min; 94 °C for 30 s, annealing temperature for 30 s, 72 °C for 20 s, 35 cycles; 72 °C for 10 min. PCR product visualization and standardization followed Gao and Li (2008b).

Microsatellite DNA diversity was characterized by allelic richness (A_R), observed heterozygosity (H_o) and expected heterozygosity (H_e). Computation of A_R , H_o and H_e was standardized to the smaller sample size at each site using the rarefaction procedure implemented in FSTAT (Goudet 2001). Genetic diversity was compared between pairwise wild and home-garden populations. Comparisons

Fig. 1 Geographic locations of seven sites in Xishuangbanna of Yunnan Province from which wild and planted *Acacia pennata* were sampled for assessment by six SSR loci. **a** geographic locations of seven sites. **b** Structure analysis results under $K = 2$

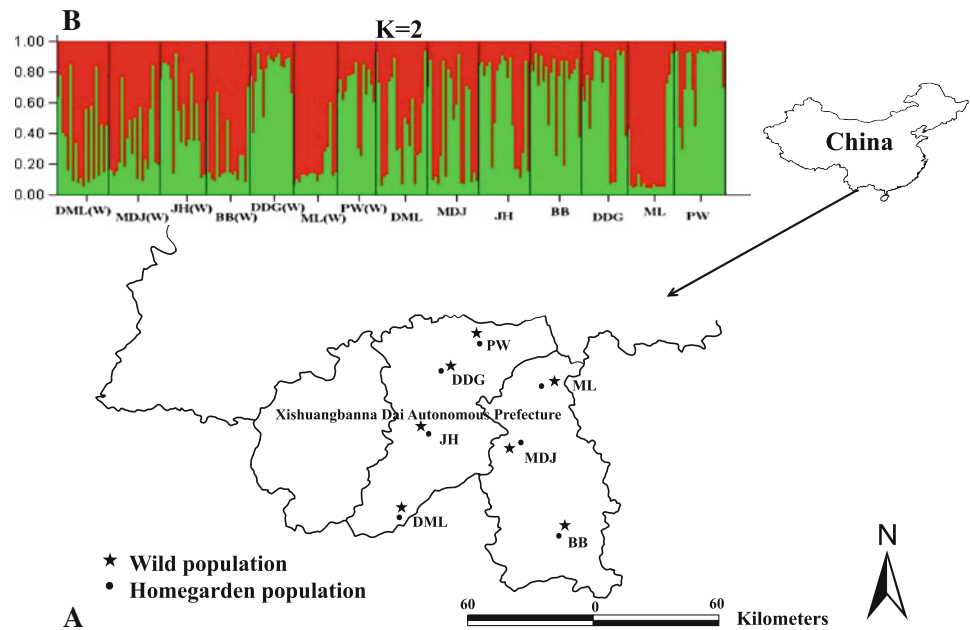


Table 1 Locations of sampled *A. pennata* populations, estimated population size, number of individuals sampled (in brackets), and distance between home-garden and wild population

Code	Locality	Wild	Home-garden	Distance (km)
DDG	Dadugang, Jinghong (22°30' N, 100° 54' E)	18 (17)	30 (18)	2.6
DML	Damenglong, Menghai (21°50' N, 100°30' E)	20 (20)	32 (20)	1.5
PW	Puwen, Jinghong (22°30'3'' N, 101°18' 5'' E)	15 (15)	29 (20)	3
JH	Menghan, Jinghong (21°54'2'' N, 100°42'5'' E)	18 (18)	27 (20)	2.7
MDJ	Menglun, Mengla (21°54'6'' N, 101°12' 3'' E)	22 (20)	34 (20)	2
BB	Bubeng, Mengla (21°30'2''N 101°18'2'' E)	23 (17)	30 (20)	1.8
ML	Manla, Mengla (22°12'6'' N, 101°30' E)	18 (17)	30 (18)	3.2
Mean		19 (17)	30 (19)	

of mean values were tested using *t*-test, and significance was taken at 0.05.

Analysis of molecular variance (AMOVA) was used to measure hierarchical genetic structure of populations using software package Arlequin v3.1 (Excoffier et al. 2005). To define and visualize the genetic relationship between home-garden and wild populations, a principal coordinates analysis (PCoA) of Nei's (1978) unbiased genetic distances between populations was performed using the program GenAlEx (Peakall and Smouse 2001), and a graph of the first two

principal coordinates was generated. Bayesian model-based clustering STRUCTURE ver.2.3 (Pritchard et al. 2000) was used to infer genetic clusters at each site. Ten runs with a burn-in of 50,000 and a run length of 500,000 iterations were performed for a number of clusters from $K = 2$ to $K = 12$. An admixture model was used and the independence of allele frequencies among populations was assumed. The most likely number of clusters was estimated according to the model value (K) based on the second-order rate of change, with respect to K , of the likelihood function (Evanno et al. 2005). To test the possible genetic drift and bottleneck events in the home-garden and wild population, bottleneck test was carried out using software BOTTLENECK (Piry et al. 1999). In the analysis, three models, Infinite Allele Model (IAM), Stepwise Mutation Model (SSM) and Two Phase Model (TPM), were tested.

Results

Genetic diversity

Allelic richness (A_R) in wild populations varied from 2.8 to 3.7 and from 2.4 to 3.8 in home-gardens (Table 3). A_R in home-garden populations was higher than that in wild populations at four sites, equal at two sites and lower at one site (Table 3). The average expected heterozygosity in home-gardens was not statistically different from that in wild populations ($H_e = 0.502$ and $H_e = 0.510$, respectively, $P > 0.05$), while the mean observed heterozygosity in home-garden populations was lower than that in wild populations ($H_o = 0.315$ and $H_o = 0.433$, respectively,

Table 2 Primer sequences, annealing temperatures (T_m) and the source of primers used for *A. pennata*

Primer	Sequence (5'-3')	T_m °C	Original code	Source species	Reference
P4	GTCGCGTACACAGACACAGT GGCGCACCTCTCTCTCTCT	50	Am367	<i>Acacia mangium</i>	Butcher et al. (2000)
P5	GGCGCAACTCTCTCTCTCTCT TTGGTCACTTAGCGCATGCC	48	Am429	<i>A. mangium</i>	Butcher et al. (2000)
P9	GAGGTAATATTTTGAATTCCTTGAAC GGTGTATACCTCTTTCTGTGG	48	AH08	<i>A. mangium</i> <i>A. auriculiformis</i>	Ng et al. (2005)
P11	CGCAACTCCATCTGATTTACTG TTATGTTGGGTTAATACGCTAACTG	46	AH18	<i>A. mangium</i> <i>A. auriculiformis</i>	Ng et al. (2005)
P13	GTGAAGGCTCTCTCTCTCTCT GGAGATGGATAGAGATGGCC	48	Ab22	<i>A. brevispica</i>	Otero-Arnaiz et al. (2005)
P18	GTCGCGTACACAGACACAGT GGCGCACCTCTCTCTCTCTCT	50	AH37	<i>A. mangium</i> <i>A. auriculiformis</i>	Ng et al. (2005)

Table 3 Comparison of genetic diversity of *A. pennata* between home-gardens and adjacent wild populations at seven sites across Xishu-angbanna, southwest China

Code	Wild populations								Home garden					
	Sample size	A_R	N_T	$N \geq 0.05$	$N < 0.05$	H_o	H_e	Sample size	A_R	N_T	$N \geq 0.05$	$N < 0.05$	H_o	H_e
DML	20	3.5	21	21	0	0.483	0.622	20	3.8	23 (21)	22 (21)	1 (0)	0.358	0.658
MDJ	20	3.7	22	20	2	0.508	0.617	20	3.7	22 (20)	22 (20)	0 (0)	0.425	0.646
JH	18	3.3	20	20	0	0.435	0.646	20	3.4	20 (18)	20 (18)	0 (0)	0.383	0.632
BB	17	3.3	21	20	1	0.51	0.6	20	3.3	22 (20)	21 (19)	1 (1)	0.467	0.587
DDG	17	2.8	18	16	2	0.52	0.57	18	3	20 (16)	17 (14)	3 (2)	0.287	0.596
ML	17	2.8	19	15	4	0.529	0.478	18	3	20 (17)	19 (14)	1 (3)	0.38	0.478
PW	15	3.1	21	18	3	0.478	0.548	20	2.4	16 (15)	16 (13)	0 (2)	0.217	0.418
Mean	17	3.2	20	18	2	0.495	0.583	19	3.2	20 (18)	19 (17)	1 (1)	0.360	0.574
Total	141	26	142	130	12	—	—	155	26	143 (127)	137 (119)	6 (8)	—	—

A_R Allelic richness (adjusted to smaller sample size in each site); N_T , Total number of alleles; $N \geq 0.05$ number of alleles with frequency equal or greater than 0.05; $N < 0.05$ number of alleles with frequency lower than 0.05. The number of alleles that were present in both wild and home-garden populations is shown in *brackets*; H_o observed heterozygosity, H_e expected heterozygosity

$P < 0.05$) (Table 3). On average, home-garden populations maintained a similar number of alleles (an average of 20 in home-garden population vs. 22 in wild population, $P = 0.890$). Of 142 alleles present in seven wild populations, 127 were also detected in home-garden populations (Table 3), implying that home-garden populations maintained at least 90 % of the total genetic variation in the wild populations. At one site (DML), the home-garden population maintained exactly the same set of alleles amplified in the wild population. At other six sites, between 71 and 95 % of alleles found in wild populations were also detected in home-garden populations. Further, of the 130 alleles with frequency greater than 0.05, 120 (92 %) were found in home-garden populations, and eight out of 12 alleles with frequency below 0.05 in wild populations were amplified in home-garden populations (Table 3). Primer P11 amplified two unique alleles with frequency of 0.53

and 0.15, respectively, in the home-garden population at site JH.

Population genetic structure and bottleneck

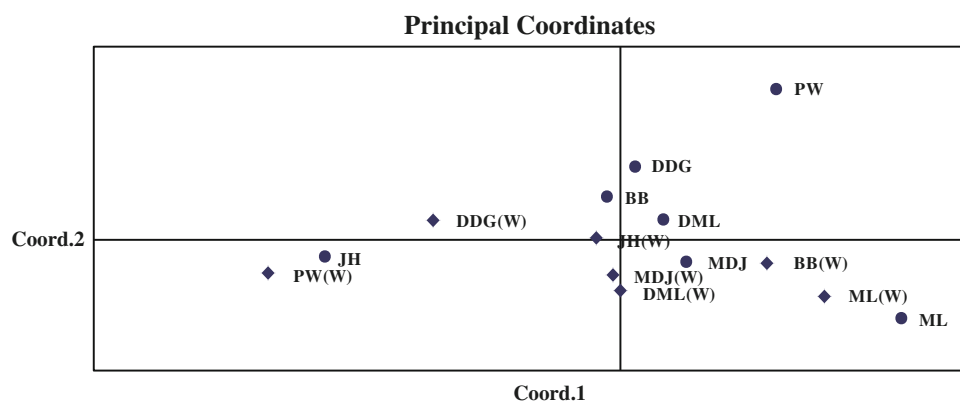
A. pennata populations in home-garden showed a higher genetic differentiation ($F_{ST} = 0.153$) than wild populations ($F_{ST} = 0.1075$). AMOVA partitioned 10.7 % of total genetic variation to between-populations, and 89.3 % to within-populations in wild population category, while those values were 15.3 and 84.7 %, respectively, for home-garden populations (Table 4). For all populations, 86.1 % of genetic variation was partitioned to within populations, 13.1 % to among populations, while only 0.8 % to between the two categories, suggesting a minimal genetic difference between wild and cultivated populations (Table 4). In principal co-ordinate analysis, populations were mixed

Table 4 Analysis of molecular variance (AMOVA) for wild, home-garden and all populations

	Source of variation	SS	Var.	PV (%)	F-statistic	P
Wild	Among populations	55.499	0.2118	10.75	0.1075	0
	Within populations	423.61	1.7577	89.25		
Home-garden	Among populations	82.907	0.3114	15.3	0.153	0
	Within populations	456.88	1.7241	84.7		
All populations	Among groups	15.958	0.0169	0.84	FSC = 0.132	0
	Among populations within groups	138.41	0.2639	13.06	FST = 0.139	0
	Within populations	880.5	1.7401	86.11	FCT = 0.008	0
	Total	1034.9	2.0209			

SS sum of squares, Var. variance components, PV percentage of variation

Fig. 2 Plot of the first two principal coordinates from a covariance matrix of Nei's genetic distance among populations of *A. pennata*. The first and second Principal Coordinates explained 34.4 % and 28.3 % of the variance, respectively. Dots represent home-garden populations, diamond for wild populations. The planted population was represented by *dot*; the wild population was represented by *diamond*



without grouping into two categories (home-garden vs wild) except the PW population (Fig. 2). Bayesian clustering analysis revealed the highest likelihood for $K = 2$ clusters (average log probability of data $\ln P(D) = -3,613$). With $K = 2$, the wild populations and its relative home-garden populations was not split into two discrete genetic clusters at each of seven sites (Fig. 1b) which means no genetic divergence between wild and home-garden population. However, bottleneck analyses have shown that four home-garden populations (DML, MDJ, JH, ML) had significant heterozygosity excess ($P < 0.05$) under one or two models, a sign of possible population bottleneck in those populations (Table 5), while three of these (DML, JH, MDJ) in wild populations also showed sign of bottleneck events.

Discussion

Agroforestry ecosystems such as home-garden may be an important resource for conservation and sustainable use of plants with economic and/or cultural values. In addition to

previous reports showing that home-garden harbor a wide range of plant species in tropical regions (Kabir and Webb 2008), here we show that home-garden populations are also capable of maintaining genetic diversity, supporting the idea that home-garden show great promise in maintaining biodiversity, and these managed patches might be of significant conservation value in tropical regions (Webb and Kabir 2009).

Empirical studies have shown a theoretical prediction that cultivated populations maintained a subset of the total genetic diversity present in their wild progenitors. Our study represents one of the few reports of its kind (Hollingsworth et al. 2005; Lengkeek et al. 2006; Miller and Schaal 2006) where wild and managed populations are matched for geographical proximity and population size and therefore provides a more realistic comparison of genetic diversity in wild and cultivated populations. In our case study, home-garden populations of *A. pennata* maintained 90 % of the genetic variation present in wild populations, and pairwise comparison within each site showed that home-garden populations were as genetically diverse as wild populations. Moreover, home-garden populations

Table 5 Bottleneck test for wild and home-garden populations under three models

Code	Wild populations			Home-garden populations			Allele frequency distribution
	IAM	SMM	TPM	IAM	SMM	TPM	
DML	0.028	0.220	0.191	0.027	0.520	0.033	Mode-shift
MDJ	0.025	0.500	0.453	0.028	0.212	0.032	Mode-shift
JH	0.023	0.041	0.028	0.032	0.557	0.206	Mode-shift
BB	0.160	0.518	0.474	0.157	0.519	0.515	L-shape
DDG	0.022	0.484	0.473	0.445	0.475	0.431	Mode-shift
ML	0.405	0.526	0.509	0.069	0.039	0.052	L-shape
PW	0.158	0.465	0.249	0.634	0.479	0.565	L-shape

P values showing heterozygosity excess under Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Model (TPM) models (Cornuet & Luikart 1996). Significant results are in bold ($P < 0.05$)

maintained over 92 % of alleles with moderate to high frequency, which is remarkable since alleles with moderate to high frequency show greater evolutionary and adaptive importance than alleles with low frequencies (Templeton 1991). Therefore, our results suggested that the home-garden populations managed by the local villagers are genetically viable as the wild populations.

The result of high genetic diversity in home-garden populations of *A. pennata* provides fresh support for the notion that human managed ecosystems, such as home-gardens and home farms, may act as refugia for biodiversity (Bhagwat et al. 2008). Many home-gardens have been established and managed since the settlement of a village and have been maintained by multiple collections from diverse wild populations over years, even generations (Long 1993). Meanwhile, wild materials were likely collected for the preferable horticultural and/or growth characters. Consequently, it is not surprising that home-garden populations maintain high genetic diversity. On the other hand, home-garden populations would be managed over long period of time, presumably at the time scale of the existence of a village and human society in the region. In other words, these home-gardens have a potential role as ex-situ conservation sites for those species with economic and/or cultural values over long period of time. Unlike other expensive ex-situ collection and conservation, local people manage their home-gardens, and are rewarded with direct improvements in their livelihood, which essentially ensures these ex-situ conserved sites will be maintained in a sustainable and continual way. Home-gardening is therefore a viable conservation option in human dominated landscapes (Webb and Kabir 2009).

Much is known that most cultivated species contain less genetic variation than their wild ancestors (Eyre-Walker et al. 1998; Shahal et al. 2003; David et al. 2006; Zhu et al. 2007; Meng et al. 2010). The reduction in genetic variation in cultivated species is probably a result of bottlenecks caused by the collection of a limited number of parents and

coupled with intense selection for agronomic traits during propagation. In our study, *A. pennata* has moderately high levels of genetic variation compared with other species with similar life history and mating system (Gao and Li 2008b). Allelic richness was sensitive to genetic bottleneck (Nei et al. 1975; Luikart et al. 1998; Hollingsworth et al. 2005). Our data showed no significant difference of allele richness between wild and home-garden populations. Bottleneck analysis showed that in seven of the studied places, three of those sites have showed sign of bottleneck events in both wild and planted populations, however, only one site in ML, the possible bottleneck in the cultivated populations might be the result of propagation from small number of material collected from wild. Our results suggested that the influence of a “cultivation bottleneck or founder effect” is possible in some home-garden populations of *A. pennata*. The result of PCoA and population clustering analysis showed that home-garden populations were not separated from their geographically approximate wild populations except only one population PW. These comments were also supported by the AMOVA analysis, which showed that only 0.8 % of the genetic variation was distributed between the home-garden and wild populations. It is therefore likely that planted populations of *A. pennata* at studied sites have been originated primarily from nearby wild populations. Re-introducing material from multiple wild populations to home-gardens may also be an ongoing process.

Conclusion

Collecting wild plants and growing them in managed patches such as home-garden for food, medicinal and cultural purposes has been an important activity in Xishuangbanna and other tropical regions (Long 1993; Long and Li 2006; Fu et al. 2006). The local people have accumulated abundant knowledge about collection, utilization, and

management of local plant resources. As an example, in surveys on two villages in Xishuangbanna, the local people have preserved as much as 65 species in their home-gardens (Long 1993, Long and Li 2006; Fu et al. 2006). The diverse collection includes many vulnerable and endangered species and also endemic species. Such practice makes human-managed patches not only important to maintain local people's livelihood and culture, but also of great value to conserve biodiversity, which was clearly demonstrated in our case study on *A. pennata*. The previously documented rich floristic diversity, along with the present result showing high level of genetic diversity of *A. pennata* in home-gardens, indicates that home-gardens are of potential significance for biodiversity conservation in human-dominated systems.

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