PLANT SPECIES BIOLOGY

Plant Species Biology (2010) 25, 43–50 doi: 10.1111/j.1442-1984.2009.00261.x

Conservation and possible reintroduction of an endangered plant based on an analysis of community ecology: a case study of *Primulina tabacum* Hance in China

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Abstract

The distribution of the rare and endangered perennial herb *Primulina tabacum* Hance is restricted to eight karst caves in southern China. To conserve *P. tabacum* and to evaluate possible reintroduction, we studied its historical distribution and conducted field surveys of both its biotic and physical environment. We used detrended correspondence analysis and canonical correspondence analysis to investigate the plant community structure and to identify the major environmental factors associated with its presence at eight sites and absence from three other sites where it formerly grew. The results revealed differences in community structure among sites and close correlations between species composition and environmental conditions. The data indicate that *P. tabacum* has special ecological requirements, including alkaline soil, low soil content of N, P, K and organic matter, low light, a high soil water content and high relative humidity, and a high atmospheric CO_2 concentration. Soil K content, relative humidity and atmospheric CO_2 concentration were the three variables most strongly associated with the vegetation composition and structure of the 11 sites. It appears that as a result of reductions in humidity, some of the historical localities of *P. tabacum* may no longer be suitable for reintroduction. *Pilea notata*, *Pteris cretica* var. *nervosa* and two moss species, *Heteroscyphus coalitus* and *Gymnostomiella longinervis*, were strongly associated with *P. tabacum*. The first two plants could be useful as indicators of suitable environments for *P. tabacum*, and the moss species could be useful as nurse plants for the reintroduction of *P. tabacum* into wild habitats.

Keywords: canonical correspondence analysis, detrended correspondence analysis, endangered plant, *Primulina tabacum*, reintroduction.

Received 29 September 2008; accepted 18 September 2009

Introduction

Primulina tabacum Hance is a calciphilous perennial herb belonging to the family Gesneriaceae. Specimens of *P. tabacum* were first collected by Andrew Henry in 1881 from Lianzhou, Guangdong, China (Hance 1883), and the species has been listed among the 'first class protected key wild plants of China' since 1999 (Peng & Chen 2002).

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Primulina tabacum has a very restricted distribution and has only been found in the limestone area of northern Guangdong and southern Hunan, China. This plant usually grows with other calciphilous and shade-tolerant plants, including bryophytes, around the entrances to deep karst caves at approximately 300 m a.s.l. (Flora of China Editorial Committee 1990). The population density of *P. tabacum* is greatest at cave entrances and then rapidly declines with distance from the entrance. In the habitat of *P. tabacum*, the soil layer is thin and nutrient poor, the concentration of CO_2 and the relative humidity are higher than in the surrounding area, and the annual air temperature ranges from 19°C to 21°C throughout the year. *Primulina tabacum* individuals grow slowly, with a maximum growth rate ≤30 g/year (Ren *et al.* 2003). *Primulina tabacum* taxonomy (Flora of China Editorial Committee 1990), ecological and biological characteristics (Ren *et al.* 2003), pollen morphology (Cao *et al.* 2007) and genetic diversity (Ni *et al.* 2006; Wang *et al.* 2009) have been investigated.

The structure, composition and dynamics of karst vegetation have received increased attention over the past 15 years (Sweeting 1995; Yuan 1997). Karst cave vegetation has unique biotic, biophysical and landscape characteristics. Plant communities in karst caves are typically composed of rare, specialized species that are adapted to low light. The biodiversity is not rich, and karst cave species cannot tolerate disturbance. Because of these characteristics, karst cave communities are very sensitive to changes in the environment; therefore, they provide early indications of environmental change (LeGrand 1973).

Local species composition and local extinctions arise partly from deterministic processes linking habitat characteristics to species-specific niches (Ozinga *et al.* 2005; Vittoz *et al.* 2006). The relationship between rare species and the environment is a fundamental ecological issue for conservation and restoration (Armstrong & Seddon 2007). However, few previous studies have investigated the quantitative relationship between an endangered species and its habitat (Jeremy 1991; Ejrnaes 2000; Wentworth & Ulrey 2000; Bowers & Boutin 2008). Relationships between a species or community and its environment or among communities are often investigated using detrended correspondence analysis (DCA), which was introduced by Hill and Gauch (1980). Canonical correspondence analysis (CCA) in combination with correspondence analysis and multi-regression analysis is also widely used to analyze relationships between species and the environment (ter Braak 1986; Leps & Smilauer 2003).

To protect this endangered species and to facilitate its reintroduction, we conducted the research described in this paper. The present study quantifies the major environmental variables that may determine the distribution and growth of *P. tabacum*. The primary questions asked in the present study were: (i) do the extant and extinct *P. tabacum* populations or their communities have different biological characteristics; (ii) what biotic and abiotic variables are responsible for the composition of *P. tabacum* communities; and (iii) how can the results be used to conserve or reintroduce *P. tabacum* to wild habitats?

Materials and methods

Study area

The study was conducted at Lianzhou City, Yongzhou County, Yanshan County and Lechang City, which collectively delimit the historical and current distribution of *P. tabacum* (24°59′–25°58′N, 111°98′–113°11′E; Fig. 1). The area is located at the boundary between Hunan and Guangdong Provinces, southern China. The climate is central subtropical monsoon, and the elevation ranges from 130 to 450 m a.s.l. The zonal original soil is lateritic, which is a heavy acidic soil. The mean annual temperature is 19.5°C and the mean annual rainfall is 1571 mm. Approximately 80% of the annual precipitation occurs in the wet season between April and October. Regional vegetation is dominated by evergreen broad-leaved forests typical of the subtropics. Representative plant families of the climax community include Lauraceae, Euphorbiaceae and Fagaceae (He *et al.* 1999).

Fig. 1 Locations of the study sites in Guangdong and Hunan Provinces, China. (A) Dixiahe Entrance, (B) Dixiahe Exit, (C) Dixiahe old Exit, (D) Zixiadong, (E) Shanbochang, (F) Dixiahe side, (G) Shangbochang side, (H) Xiaguanchun, (I) Xiaobeijiang, (J) Yangshan edge and (K) Jingjiling.

Field sampling and laboratory analyses

We examined all *P. tabacum* specimens deposited in the herbaria of the Royal Botanical Gardens, Kew; the Institute of Botany, Chinese Academy of Sciences; the Guangxi Institute of Botany, Chinese Academy of Sciences; the Kunming Institute of Botany, Chinese Academy of Sciences; and the South China Botanical Garden, Chinese Academy of Sciences. These are the major herbaria containing collections of material from southern and central China. The *P. tabacum* specimens were collected in 1881, 1912, 1936, 1983, 1986, 1991 and 2004. In addition, we used the Flora of China (Flora of China Editorial Committee 1990) and the Flora of Guangdong (Wu 2005) for clarification and confirmation.

Based on the site descriptions from the herbarium specimen records and local floras, we established 34 quadrats during 2002–2004 at 11 cave study sites (three quadrats per site, except for one site where four quadrats were established because it had four different microhabitats) to compare the vegetation among sites and to elucidate the environmental factors. The longitude, latitude and elevation were recorded with a Magellang GPS, Smyrna, TN, USA). Because of the small size of the caves and *P. tabacum* habitats (<4 m × 8 m per site), only three quadrats (1 m × 1 m) were sampled along the gradient starting at the cave entrance and extending into deep caves where *P. tabacum* grew. In each quadrat, the identity, height, density and crown of all plant species were recorded. For mosses, the name and coverage area were recorded. To estimate the coverage of mosses, we used a 1 m × 1 m frame with small panels (5 cm × 5 cm). The vegetation surveys were conducted in April 2003, October 2005, October 2007 and April 2008. These four sets of data were similar, and we only used the data from October 2007.

We collected soil samples in October 2007. Because the soils around the cave entrances were very thin, soil samples were randomly collected from five points in each quadrat using a 5-cm diameter soil corer to a depth of 0.5–2.1 cm. A total of 2.5 kg of soil was collected from each quadrat, and composite soil samples of approximately 0.5 kg per quadrat were used for laboratory analysis. The soil subsamples were air-dried and sieved for analysis of soil chemical characteristics, including water content; pH; N, P and K content; and soil organic matter content (Standford & English 1949; Olsen *et al.* 1954; Institute of Soil Science, Chinese Academy of Sciences 1978; MEWAM 1986). There were three replicates per site (i.e. one replicate per quadrat). Soil water content was also measured in April 2008.

We measured the photosynthetically active radiation (PAR), CO_2 concentration, air temperature and relative humidity using a Li-6400 Photosynthesis System and its accessories (Li-COR, Lincoln, NB, USA) at each site on 1 day in July 2007, October 2007, January 2008 and April 2008. Measurements were made hourly between 08.00 hours and 18.00 hours. The average values of relative humidity, PAR, CO_2 concentration and air temperature were calculated for each site on each day.

Data analysis

The Shannon–Weiner diversity index (Lambshead *et al.* 1983) was calculated using the Biodiversity Professional Program Packet 2.0 (Natural History Museum and the Scottish Association for Marine Science, London, UK). The species importance values (IV) in each sample were calculated using the following formula:

$$IV = (\text{relative density} + \text{relative frequency} + \text{relative dominance})/3,$$

where the relative frequency is the percentage of the quadrat containing a plant species over the total number of quadrats at the same site, and the relative dominance is the sum of the relative crown size of a shrub or herb species within a quadrat. The IV value of moss was estimated by the coverage and frequency (Ren *et al.* 2003).

We first used the IV of all plant species in different quadrats to construct a vegetation matrix that included the 34 quadrats and 23 plant species recorded at the 11 sites. We also built a data matrix for the 10 biotic and abiotic variables characterizing each site. Detrended correspondence analysis and CCA were used to quantify and describe the community structure with PC-ORD4 for Windows (Hill & Gauch 1980; ter Braak 1986; Økland 1990; Girard *et al.* 2002; Lachance & Lavoie 2008). Major gradients in vegetation composition were identified using DCA. Canonical correspondence analysis was then used to examine the relationships between vegetation gradients and abiotic variables (Leps & Smilauer 2003). In addition, multiple stepwise analysis and ANOVA were conducted using the SPSS 10.0 software package (Anon 2000).

Results

Distribution of P. tabacum *populations*

Based on the records of herbarium specimens, we established that *P. tabacum* has been known from only 11 sites: all sites are karst cave sites located on the boundary between Guangdong and Hunan Provinces. Based on our field work at these 11 locations and additional historical information, however, we determined that *P. tabacum* existed at only eight sites in 2003–2007 (A, C–I in Fig. 1) and had disappeared from the other three sites (B, J and K). At the eight sites where *P. tabacum* occurred, plants grew at the cave entrances where the soil was moist and the relative humidity was high. The environmental conditions at site I, which was located at a cave beside a river, differed somewhat from the conditions at the other seven sites with *P. tabacum*. At the three sites where *P. tabacum* had disappeared, the soil water content and relatively humidity were low (Fig. 1; Table 1).

Comparison of vegetation among the sites

A total of 23 plant species were recorded in the 34 quadrats, indicating that overall species diversity at the sites was low (Tables 1,3). However, the number of plant species was greater at sites where *P. tabacum* grew than at nearby sites where *P. tabacum* was absent. Thus, more than five plant species were found at sites C, D, E, G and I, where *P. tabacum* occurred, but only 1–2 species grew at sites B, J and K, where *P. tabacum* was absent.

Table 1 Plant species and the number of individuals of each species observed at the 11 study sites (A–K: see Fig. 1) in Guangdong and Hunan Provinces

Code	Name	No. individuals										
		A	B	C	D	E	F	G	H	I	J	K
S3	***Primulina tabacum* Hance**	**157**	**0**	**10**	**50**	**79**	**45**	**144**	**22**	**7**	**0**	**0**
S1	***Pilea notata* C. H. Wright**	**7**	**0**	**2**	**6**	**25**	**22**	**0**	**0**	**0**	**0**	**0**
S2	***Lindasea orbiculata* (Lam). Mett**	**4**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S4	***Pteris cretica* Linn.var.nervossa (Thunb.) Ching et S.H.Wu**	**1**	**0**	**13**	**8**	**0**	**9**	**1**	**7**	**0**	**0**	**0**
S5	***Selaginella uncinata* (Desv.) Spring**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S6	***Oplismenus compositus* (Linn.) Beauv**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S7	***Carex cryptostachys***	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S8	***Elatostema rupestre* (Buch.-Ham.) Wedd**	**0**	**0**	**0**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S9	***Malaisia scandens* (Lour.) Planch**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S10	***Asplenium coenobiale* Hance**	**0**	**0**	**0**	**22**	**0**	**0**	**0**	**0**	**9**	**0**	**0**
S11	***Heteroscyphus coalitus* (Hook.) Schiffn**	✔	**0**	✔	✔	✔	✔	✔	✔	✔	**0**	**0**
S12	***Adiantum capillus-veneris* Linn**	**0**	**0**	**0**	**3**	**1**	**0**	**15**	**0**	**0**	**0**	**0**
S13	***Viola verecumda* A. Gray**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**
S14	***Arthraxon hispidus* (Trin.) Makino**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
S15	***Pueraria phaseoloide* (Roxb.) Benth**	**0**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**3**
S16	***Impatiens balsamina* Linn**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**
S17	***Boehmeria nivea* (Linn.) Gaudich**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**0**
S18	***Paspalum conjugatum* Berg**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**0**	**0**
S19	***Gymnostomiella longinervis***	✔	**0**	✔	✔	✔	✔	✔	✔	✔	**0**	**0**
S20	***Begonia fimbristipula* Hance**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**29**	**0**	**0**
S21	***Trachelospermum jasminoides* (Lindl.) Lem**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**
S22	***Selaginella tamariscina* (P. Beauv.) Spring**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**		**0**
S23	***Ophiorrhiza cantoniensis* Hace**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**		**0**
	Total number of species	**4**	**1**	**5**	**10**	**5**	**3**	**5**	**4**	**7**	**2**	**1**

✓, presence of moss.

The major vascular plant species that coexisted with *P. tabacum* were *Pilea notata* C.H. Wright and *Pteris cretica* L. var. *nervosa* (Thunb.) Ching et S.H.Wu. The communities in which *P. tabacum* grew also included several moss species, such as *Heteroscyphus coalitus* (Hook.) Schiffn and *Gymnostomiella longinervis* Broth. Each of the studied communities without *P. tabacum* included *Pueraria phaseoloides* (Roxb.) Benth., but not *H. coalitus* or *G. longinervis*. The pattern of species diversity among sites (Shannon–Weiner diversity index in Table 3) was similar to the pattern for species number (Table 1). The communities at sites C, D and I, where *P. tabacum* was present, had a richer biodiversity than the communities at sites B, J and K, where *P. tabacum* was absent. The species diversity at the other sites (A, E–H) was intermediate.

In DCA ordinations, the relative position of the 11 communities on the axes provides a measure of their floristic similarity. Figure 2 shows a plot of the first and second axes in the DCA of the vegetation composition in the 34 quadrats at the 11 study sites. The first axis explained approximately 13.6% of the total variation in floristic composition among sites (Table 2). In addition, the first axis had strong correlations with the environmental factors ($r = 0.831$). The quadrats were grouped into three clusters (Fig. 2). The first cluster consists of the three quadrats at site I and is located on the left side of the graph. The second cluster is the largest and consists of many quadrats at the seven sites where *P. tabacum* was present (A, C–H).

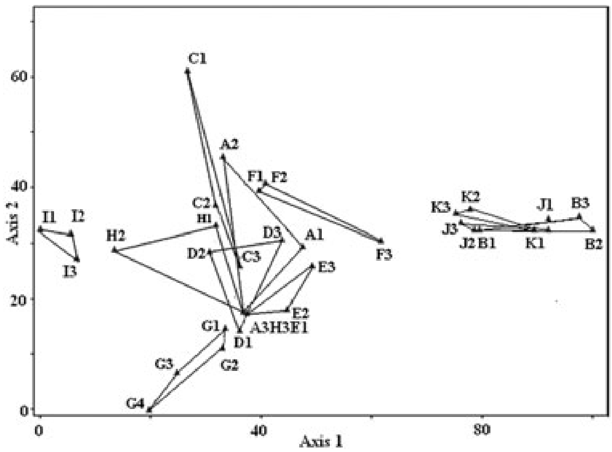

Fig. 2 Detrended correspondence analysis of the vegetation from the 34 quadrats at the 11 study sites. The letters (A–K) designate the sites (see Fig. 1) and the numbers 1–4 represent the quadrat number at each site.

Table 2 Summary statistics and comparisons of the analytical results between canonical correspondence analysis and detrended correspondence analysis

Axis	1	2	3	4
Eigenvalue				
CCA	0.652	0.426	0.291	0.275
DCA	0.706	0.350	0.176	0.070
Species–environment correlation coefficients				
CCA	0.842	0.802	0.766	0.765
DCA	0.831	0.827	0.736	0.868
Cumulative variance in species composition (%)				
CCA	13.3	22.0	27.9	33.5
DCA	13.6	20.3	23.8	25.2
Cumulative variance in species–environment correlations (%)				
CCA	28.0	46.3	58.7	70.5
DCA	28.5	39.2	0.0	0.0

The sum of the eigenvalues is >1 because the ratio of the total number of quadrats (34) and total number of environmental factors (10) is only 3.4; in general, if the ratio is >5, the sum of the eigenvalues is <1. However, the Mont Caro test *P*-value is <0.05, indicating that the results are valid. CCA, canonical correspondence analysis; DCA, detrended correspondence analysis.

The third cluster is located on the right side of the graph and consists of quadrats at sites where *P. tabacum* did not grow (B, J, K).

Relationships between environmental factors and vegetation

Soil pH was similar among the 11 sites and ranged from 7.66 to 7.80 (Table 3). Temperature was also similar among the sites, and the CO_2 concentration ranged from 382.24 to 400.72 p.p.m. The soil layer at the karst cave entrance was very thin. The soil water content at each site was extremely low in October (<1%) and high in April (7.0–9.3%, except for 0.93% at site A) (Table 3). The contents of N, P, K and organic matter were lower in the soils at the 11 sites than in the regional soils (He *et al.* 1999). The PAR density at sites A–I was low and varied between 49.3 and 85.3 μmol/m²/s; however, the PAR densities at sites J and K were higher than those at the other sites. The relative humidity was slightly lower at sites I, J and K compared with the other sites (Table 3).

In the diagram showing the CCA analytical results (Fig. 3), the length of the environmental factor line represents the degree of correlation with vegetation composition, and the angle and direction of the line represent the relationship. The successive decrease of the eigenvalues of the first four axes (Table 2) suggests a well-structured dataset. The percentage variance (%) explained by the first axis was very close to the case of the first axis in the DCA (13.3 *vs* 13.6), and the species–environment correlation

Table 3 Species diversity and related ecological variables (mean ± standard devaition) at the 11 sites (A–K)

Site	Shannon–Weiner index	Soil water content (%) in October	Soil water content (%) in April	pH	P (p.p.m.)	K (p.p.m.)	Soil organic matter (%)	N (%)	PAR (μmol/m²/s)	Temperature (°C)	Relative humidity (%)	$[CO_2]$ (p.p.m.)
A	0.3193	0.93 ± 0.15	8.83 ± 0.12	7.66 ± 0.12	105.93 ± 64.10	270.18 ± 63.01	3.77 ± 0.37	0.05 ± 0.03	65.0 ± 40.1	20.3 ± 0.1	96.0 ± 2.0	398.24 ± 32.21
B	0.6365	0.70 ± 0.38	7.53 ± 0.13	7.68 ± 0.08	77.27 ± 1.08	87.15 ± 5.12	1.84 ± 0.63	0.02 ± 0.00	86.3 ± 12.7	20.2 ± 0.2	85.3 ± 1.0	364.00 ± 8.12
C	1.3249	0.81 ± 0.02	8.76 ± 0.22	7.72 ± 0.05	51.98 ± 2.81	341.84 ± 31.89	2.82 ± 0.41	0.02 ± 0.01	60.3 ± 40.1	20.2 ± 0.1	97.0 ± 2.0	400.36 ± 44.59
D	1.7294	0.75 ± 0.11	9.11 ± 0.09	7.74 ± 0.06	89.87 ± 17.69	163.63 ± 11.06	4.68 ± 1.17	0.06 ± 0.02	49.3 ± 6.1	19.7 ± 0.2	95.7 ± 0.6	400.19 ± 29.16
E	0.8123	0.91 ± 0.02	8.93 ± 0.11	7.74 ± 0.05	49.07 ± 1.93	260.91 ± 4.78	5.77 ± 1.71	0.07 ± 0.01	59.7 ± 9.0	19.9 ± 0.4	95.0 ± 1.0	382.24 ± 17.73
F	0.9792	0.78 ± 0.01	9.28 ± 0.21	7.72 ± 0.01	80.16 ± 1.81	185.66 ± 9.30	3.60 ± 0.02	0.05 ± 0.01	63.0 ± 45.01	20.4 ± 0.3	84.3 ± 10.3	400.72 ± 32.78
G	0.6254	0.70 ± 0.03	8.97 ± 0.09	7.71 ± 0.04	60.99 ± 8.91	183.73 ± 40.18	3.27 ± 0.80	0.03 ± 0.00	85.3 ± 7.7	20.4 ± 0.1	90.25 ± 1.7	385.21 ± 9.12
H	0.9933	0.68 ± 0.02	8.84 ± 0.07	7.71 ± 0.03	88.12 ± 7.24	100.26 ± 31.21	3.48 ± 0.37	0.05 ± 0.02	85.3 ± 8.4	19.4 ± 0.2	92.0 ± 1.3	389.98 ± 1.12
I	1.5154	0.75 ± 0.01	9.30 ± 0.02	7.73 ± 0.05	89.31 ± 2.17	102.31 ± 27.81	1.65 ± 0.84	0.03 ± 0.01	83.6 ± 4.1	19.8 ± 0.2	81.1 ± 2.3	383.23 ± 23.23
J	0.8676	0.55 ± 0.01	7.00 ± 0.04	7.80 ± 0.03	49.38 ± 4.38	89.88 ± 12.27	1.25 ± 0.10	0.02 ± 0.01	124.0 ± 22.3	20.2 ± 0.1	75.7 ± 9.3	372.21 ± 28.72
K	0.5623	0.59 ± 0.27	7.09 ± 0.07	7.70 ± 0.14	54.38 ± 5.31	78.35 ± 7.69	2.01 ± 0.29	0.02 ± 0.01	114.3 ± 3.1	20.4 ± 0.1	72.4 ± 3.2	379.91 ± 56.15

PAR, photosynthetically active radiation.

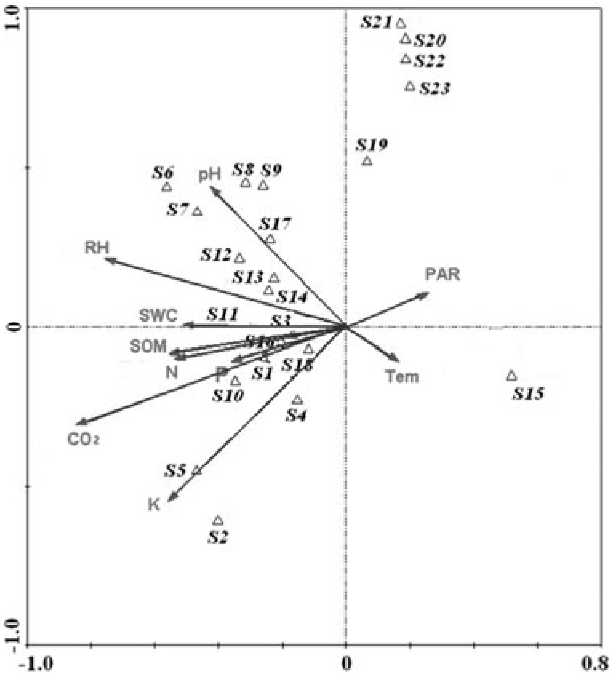

Fig. 3 Canonical correspondence analysis of the vegetation and 10 environmental factors in 34 quadrats at the 11 study sites. CO_2, concentration of CO_2; K, kalium content; N, nitrogen content; P, phosphorus content; PAR, photosynthetically active radiation; pH, pH value; RH, relative humidity; SOM, soil organic matter; SWC, soil water content; Tem, air temperature. The species codes (S1–S23) are explained in Table 1.

was only slightly higher with CCA than with DCA. These analyses indicate a strong correlation between vegetation and environmental variables and suggest that environmental variables could be responsible for variations in species composition. The species–environment correlations were higher for the first three canonical axes, explaining 61.3% of the total cumulative variance.

The correlations between the CCA species ordination scores and the environmental variables measured for each plot are shown in Table 4. *Primulina tabacum* (s3), *P. notata* (s1) and *P. cretica* var. *nervosa* (s4) were closely related to soil water content, soil organic matter, N content and relative humidity. *Pueraria phaseoloides* (s15) was strongly correlated with PAR density.

Relationships between environmental factors and species diversity

Multiple stepwise analysis indicated that, among the environmental factors studied, relative humidity was significantly related to species diversity as measured by the Shannon–Weiner diversity index ($y = -0.1277 + 0.015\text{RH} + 0.005\text{P} + 0.004\text{CO}_2$; $r = 0.492$, $P = 0.01$). The IV of *P. tabacum* was positively related to each of the following

Table 4 Correlation between the environmental matrix and the first two axes of the canonical correspondence analysis of the species ordination scores

Variable	Axis 1	Axis 2
SWC	–0.430	0.211
pH	0.479	0.046
P	–0.185	0.033
K	–0.636	–0.229
SOM	–0.381	0.306
N	–0.355	0.255
PAR	0.299	–0.313
Tem	–0.109	0.132
RH	–0.107	–0.074
CO_2	–0.716	0.046

CO_2, concentration of CO_2; K, kalium content; N, nitrogen content; P, phosphorus content; PAR, photosynthetically active radiation; pH, pH value; RH, relative humidity; SOM, soil organic matter; SWC, soil water content; Tem, air temperature.

environmental factors: CO_2 concentration ($r = 0.661$, $P < 0.001$), relative humidity ($r = 0.492$, $P < 0.01$), soil organic matter ($r = 0.392$, $P < 0.05$), P content ($r = 0.388$, $P < 0.05$), K content ($r = 0.374$, $P < 0.05$) and N content ($r = 0.372$, $P < 0.05$).

A one-way ANOVA showed a strong correlation between the moss species, *H. coalitus* or *G. longinervis*, and *P. tabacum* ($F = 203.294$, $P < 0.001$).

Discussion

As an endangered and rare plant, *P. tabacum* currently exists at only eight karst cave sites along the boundary between Guangdong and Hunan Provinces, China. In contrast to those eight sites, the three sites where *P. tabacum* has disappeared (sites B, J, and K) have low plant diversity and are located near each other. In contrast, the communities at sites A, C, D, E, F, G and H are physically separate and occupy different environments, suggesting greater differentiation in species composition and thus higher species diversity.

Primulina tabacum was only found in alkaline soils with poor nutrition and apparently prefers low light conditions and relatively wet habitats. *Primulina tabacum* might be intolerant to highly fluctuating temperatures. In addition, atmospheric concentrations of CO_2 were relatively high at the sites where *P. tabacum* grew.

A CCA analysis indicated that the soil K content, atmospheric CO_2 concentration and relative humidity were the three environmental variables most closely associated with the composition and structure of vegetation at all sites. Associations between the composition and structure of the plant community and environment were also strong for soil pH, soil water content, N content and soil organic matter. In the CCA diagram, the length and angle of each factor's line and the proximity of each species to the lines illustrate these relationships.

The present study identified differences in the plant communities between sites where *P. tabacum* still grows and where it no longer grows. This study also determined associations between the vegetation composition and structure of sites with and without *P. tabacum* and environmental factors. These findings may have important implications for the conservation and reintroduction of *P. tabacum*.

Primulina tabacum generally grows in cave entrances with unique microenvironmental conditions, and even minor changes in these special conditions could affect the *P. tabacum* population. For example, He and Li (2005) reported that the entrance to the Dixiahe cave (0.065 ha) supported a *P. tabacum* population of approximately 900 individuals in 1998. Our investigation showed that the population at this site (site A) had declined to 613 individuals in 2007. Considering the changes in habitat where *P. tabacum* has disappeared (as documented in the present study), we suspect that the relative humidity at the Dixiahe entrance has gradually declined, probably because of increased tourism in the past several years. We therefore suggested that tourism managers increase the humidity by creating misting displays at cave entrances (H. Ren, pers. comm., 2007); the population size of *P. tabacum* has gradually increased since 2009 (H. Ren, unpubl. data, 2009).

Cave plants are adapted to the specialized environments of caves (Bar 1967) and the isolation of the eight extant populations may result in *P. tabacum* polarization. Cao *et al.* (2007) reported that the morphology of *P. tabacum* at site d was different from that at the other sites. Nevertheless, a better understanding of the principal environmental variables affecting the survival of *P. tabacum* will help guide reintroduction. Our results suggest that low soil water content and low relative humidity could partly explain why *P. tabacum* was absent from sites B, J and K. The present study also indicates that some of the historical sites are not suitable for reintroduction. When a cave entrance is being assessed as a possible site for reintroduction, close consideration of soil water content, soil organic matter, N content, relative humidity and other ecological factors could improve the survival rate of *P. tabacum* individuals.

For *P. tabacum* reintroduction, *P. notata* and *P. cretica* var. *nervosa* could be used as indicator species because their presence signals that the habitat is suitable for *P. tabacum*. Considering the close relationship between *P. tabacum* and the mosses *H. coalitus* and *G. longinervis*, mosses might act as nurse plants for *P. tabacum* reintroduction. In initial trials using the information available, we have recently made some successful reintroductions of tissue-cultured *P. tabacum* seedlings to a wild cave entrance (Ma *et al.* 2007).

According to China's newly established strategy for plant conservation, 10% of endangered plants in China will be reintroduced to wild habitats (China's Strategy for Plant Conservation Editorial Committee 2008). Because *P. tabacum* was listed as one of these high-priority species, more data and information about the genetics, life history and habitat requirements of this species are critically needed.

Acknowledgments

We thank Guowei Chu for nutrient element analyses and Shijin Li, Guohua Ma, Wenjun Duan, Xiangxu Huang and Changxin He for assistance in fieldwork. We are grateful to Dr J. Faclone for valuable comments on the manuscript. We also thank Professor Bruce Jaffee for polishing the English. This research was financially supported by the National Basic Research Program of China (2009CB421101, 2007CB411600) and the Guangdong and Guangzhou Sci-Tech Planning Project (2006B60101034, 2008A060207017, 2007J1-C0471).

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