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ORIGINAL PAPER

Surprisingly high orchid diversity in travertine and forest areas in the Huanglong valley, China, and implications for conservation

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Abstract The presence of such a large number of terrestrial orchid species in a small area (ca. 1 km^2) of the Huanglong valley in southwestern China is uncommon for this country. Studying the relationship between the distribution patterns of these orchid species and their microenvironments may help us understand this uncommon phenomenon. We established 662 1 m × 1 m plots, measured the cover of each species and found that there were 33 orchid species distributed mainly in two different habitats, i.e. travertine areas and forest. In the travertine areas, 30 orchid species were found; the six most common ones being *Cypripedium bardolphianum*, *Cypripedium flavum*, *Cypripedium tibeticum*, *Galearis diantha*, *Ponerorchis chusua* and *Phaius delavayi*. However in the forested habitat, we found 21 orchid species; the most common ones being *Tipularia szechuanica* and *Goodyera repens*. Travertine areas had a higher number of orchid species as well as higher numbers of orchid species per plot as compared to forest. Light availability seems critical to the performance and distribution of orchid species. Stream flow through the travertine area during the

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orchids growing season appears to be an important factor in shaping and maintaining stable microenvironments favorable to the growth and reproduction of orchids. The results presented in this study suggest that some orchid species in the travertine area might be threatened if the travertine stream flows were to change or be disrupted.

Keywords Calcareous soil · Diversity · Light availability · Microenvironment · Stream flow · Terrestrial orchid

Introduction

Environmental factors have been shown to have a significant impact on species richness and diversity in plant communities (Crawley 1990; Pausas and Austin 2001; Dupré and Ehrlén 2002; Schaffers 2002; Maestre 2004). Light, water and nutrient availability are important environmental factors which could influence plant growth and thus affect reproduction and population performance (Arntz 1999).

Orchideaceae (the orchid family) is one of the largest families of flowering plants. There are about 20,000 to 35,000 orchid species in the world (Dressler 1993; Mabberley 1997). However, many orchid species are rare and have a limited distribution (Dressler 1993; Mabberley 1997; Brzeskiewicz 2000; Cribb et al. 2003). This is likely because each orchid species has developed particular requirements for reproductive success (Kull 1995, 1998; Brzeskiewicz 2000; Johnson 2000; Pellegrino et al. 2005; Tremblay et al. 2005) and/or tolerances for specific environmental characteristics (e.g. acidity, texture, moisture, available minerals and temperature) (Brzeskiewicz 2000). Therefore, an orchid species will only become established in the place where these specific requirements are met (Brzeskiewicz 2000).

Availability of suitable microsites seems to be the principal factor that limits reproduction and thus the spread of some orchid species (Willems and Bik 1991; Calvo 1993; Kull 1998). Kull (1998) reported that suitable sites for seedling establishment of *Cypripedium calceolus* in Europe should have sparse vascular plant cover and more intense light, but more extensive moss cover and more moisture. Brzeskiewicz (2000) reported that the seeds of *Cypripedium arietinum* may not germinate because unsuitable conditions (e.g. soil water content). Dependence on mycorrhizal fungi for seed germination is an important component of the orchid life cycle (Arditti et al. 1990; Rasmussen and Whigham 1998; Brundrett et al. 2003), and availability of mycorrhizal fungi is closely related with microsites which will in turn limit the distribution of orchid species (Clements 1987; Tremblay et al. 1998; Batty et al. 2001).

However, much of the previous work relative to orchids in China has been focused on taxonomy and biogeography (Chen and Luo 2003; Luo et al. 2003). A few pollination biology studies have been reported in recent years (e.g. Luo and Chen 1999; Banziger et al. 2005; Li et al. 2006; Sun et al. 2006). Therefore, there is a wide gap in our knowledge regarding ecological studies of Chinese orchids, e.g. the characteristics of distribution, habitats, population and demography etc. (Luo et al. 2003; Huang et al. 2007).

Because of many challenges in orchids reproduction, seed germination, seedling establishment, and growth, high orchid diversity within small geographic areas is uncommon in China. One such area is found within an area of about 1 km^2 in the Huanglong valley where some orchid species dominate the herb layer (Li et al. 2005). The orchid species in the Huanglong valley are distributed mainly in two different habitats, i.e. in travertine-depositing areas and in forest understories. The travertine-depositing area (called travertine area hereafter) is a bed of layered flowstone, a calcium carbonate rock continuously being deposited from the distinctive streams bubbling from a spring at the head of the valley (Perner and Luo 2007). The woody plant community in the travertine area consists of sparse coniferous trees or open shrubs or both and is considered to be a semi-open environment. The forested area has dense overstory and is shaded (Li et al. 2005).

To understand which environmental factors contribute to orchid species diversity in the Huanglong valley, we conducted a field study between June and August in 2005. Our primary objectives were to address the following questions: (1) Are there differences in the distribution patterns of orchid species between the semi-open travertine areas and forested habitats; and (2) What is the relationship between the orchid species distribution and site characteristics?

Site description

The Huanglong valley is located within the Huanglong National Park (32°41′–32°54 N, 103°44′–104°3 E), one of the Natural World Heritage Sites. The most attractive area of the Park is the 3.5-km-long and up to 300 m-wide travertine structure at altitudes of 3,100 to 3,570 m (Ran and Liu 2002). Mean annual temperature and mean annual precipitation are 5–7°C and 730–760 mm respectively, and most rainfall (70–73% of the annual precipitation) occurs between May and September (Ran and Liu 2002).

Much of the travertine area is unvegetated especially where streams flow from April to November every year but some other parts which are less frequently flooded, are semi-open with sparse coniferous trees or shrubs or both, which are considered to be clear indicators of high light, low shade environments. Common tree species in this community include *Abies ernestii*, *Abies faxoniana*, *Betula utilis*, and *Salix tetrasperma*; common shrubs are *Berberis polyantha*, *Lonicera szechuanica*, and *Sorbus hupehensis*. The herb layer includes *Allium prattii*, *Arctous ruber*, *Carex lehmanii*, *Elymus nutans*, *Gentiana scabra*, and *Polygonum macrophyllum*, as well as orchid species (Li et al. 2005). Most orchid species occur within an area of the travertine that is about 2 km long and 100–300 m wide at altitudes between 3,100 m and 3,250 m. Some orchid species occur within a nearby patch of coniferous woodland, about 200 m in width and 300 m in length, at 3,200 m (hereafter called forest area) which once had travertine stream flow that ended after a plank road was constructed several hundred years ago (Kou Y. personal communication). *Abies fabri* and *Picea asperata* are the dominant overstory species in this community, while *Carex lehmanii*, *Oxalis acetosella* spp. *griffithii*, *Pedicularis humilis*, *Polygonum macrophyllum*, *Rhododendron watsonii*, *Ribes* sp. and *Rosa murielae* are common herbs and shrubs.

Methods

Between June and August, 2005, understory vascular plants (including seedlings and saplings of trees and shrubs) were surveyed in 662 1 m × 1 m randomly selected plots in both the travertine (554 plots) and forest areas (108 plots). Numbers of individuals (single shoots) of each species were counted (numbers of individual of each species in Gramineae and Cyperaceae were not recorded due to the difficulty in distinguishing single individuals). Percent cover of each species, total combined understory vegetation, moss cover, canopy (tree) cover, and shrub cover were also estimated. Soil depth (cm) and elevation of each plot was recorded. Habitat patches of less than 1 m × 1 m were not sampled.

Frequency, average cover and importance value index (IPI) of all orchid species as well as of other species (not provided in this paper) in the plots were calculated and tabulated. Richness was calculated for plots in travertine and forest areas (total number of species, average number of species per plot, and orchid species). IPI was calculated as (relative frequency + relative cover)/2.

We chose a clear day in June (June 27, 2005) to assess environmental factors both in a typical representative part of the travertine habitat which had one isolated coniferous tree with shrubs, and in the forest. Light intensity at about 35 cm above the ground was measured using a light meter (TES-1339) hourly from 9:00 to 18:00 on that day. Light intensity was also measured in a fully open site in order to calculate relative light intensity. Relative light intensity was calculated from values measured in representative part of the travertine area or in the forest divided by the values measured in the fully open site. Air temperature, soil temperature and relative humidity were measured concurrently using a humidity temperature meter (CENTER-314).

Thirty soil samples (five on 27 June 2005, and 25 on 24 August 2007, respectively) were collected in each of the two habitat types. Soil humidity was calculated as the percent difference between fresh soil and that dried to constant mass at 105°C; Organic matter was measured by loss-on-ignition (ashing); N was determined by the Kjeldahl method; Ca, K, and P were assessed using atomic absorbtion spectrophotometry (AAS, GBC9932AA) and soil pH was measured from 1:5 soil:water extracts.

Data analysis

Student's *t* was used to test the differences in soil, biotic, and vegetation characteristics between the travertine and the forest area. Correlative relationships of the density of eight common orchids (plants/plot) and percent cover of trees, shrubs, herbs, and moss were performed using the Spearman Rank test. Differences in frequency of occurrence of orchid species in the travertine and forest areas were tested using the Chi-Square test. SPSS Version 11.5 for Windows was applied for all tests.

Results

Site characteristics

Tree cover and moss cover were significantly lower in the travertine area than in the forest area, but shrub and herb coverage were higher (Table 1). Based on the 27 June 2005 assessment, air temperature, soil temperature, and relative light intensity were significantly higher in the travertine area than in forest (*t*-test: $P = 0.016$, $P < 0.001$ and $P = 0.004$ for air temperature, soil temperature, and relative light intensity, respectively) (Fig. 1a, b, d), but relative humidity was significantly lower (*t*-test: $P = 0.001$) (Fig. 1c). In Fig. 1d, relative light intensity in the travertine area is shown to drop to low levels from noon onwards which is due to progressive shading of the assessment location by the single tree and shrubs.

The total calcium and pH values were much higher in the travertine area, whereas in the forest area the moisture, organic matter, total nitrogen, and soil depth were higher (Tables 1, 2). However, the total potassium and total phosphorus showed no difference between the two habitats (Table 2).

Table 1 Vegetation traits (a), biotic conditions (b) and soil depth (c) in the travertine and forest area in Huanglong valley

	Travertine area		Forest area	
	Mean (SE)	Max/Min	Mean (SE)	Max/Min
(a) Vegetation traits	*N* = 554		*N* = 108	
No. of all species	109		81	
No. of orchid species	30		21	
No. of all species per plot (Ns)	8.12(0.10)	15/1	8.04(0.20)	13/2
No. of orchid species per plot***	3.23(0.06)	9/1	2.37(0.11)	5/1
(b) Biotic conditions	*N* = 554		*N* = 108	
Tree cover (%)***	27.19 (1.24)	100/0	84.12 (1.07)	95/0
Shrub cover (%)***	44.76 (1.23)	100/0	8.87 (1.67)	90/0
Herb cover (%)***	59.12 (0.96)	100/1	27.53 (1.89)	100/3
Moss cover (%)***	87.77 (1.0)	100/0	96.32 (1.2)	100/8
(c) Soil depth	*N* = 554		*N* = 108	
Soil depth (cm)**	8.12(0.31)	40/0.5	23.56(1.17)	55/3

N = sample size. Characteristics that were significantly different between travertine and forest are indicated with asterisks (*t*-test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Ns = not significant)

Richness and orchid species

We recorded 124 understory plant taxa in the 662 study plots, including 33 orchid species of 20 genera (Table 1). We found 30 orchid species in the travertine area, of which six (*Cypripedium bardolphianum*, *C. flavum*, *C. tibeticum*, *Galearis diantha*, *Phaius delavayi* and *Ponerorchis chusua*) were common (i.e. frequency >37%: Table 3). In the forest area, 21 orchid species were recorded, the most common ones being *Tipularia szechuanica* and *Goodyera repens*. Twelve orchid species (*Amitostigma faberi*, *Coeloglossum viride*, *Cypripedium guttatum*, *C. calcicolum*, *C. tibeticum*, *Epipactis helleborine*, *Galearis roborowskii*, *Gymnadenia orchidis*, *Herminium monorchis*, *Neottianthe monophylla*, *Galearis* sp., and *Platanthera minor*) were found only in the travertine area and three (*Goodyera wolongensis*, *Listera biflora*, and *Platanthera fuscescens*) only in the forest area.

The number of species per plot did not differ between the two habitats ($t = 0.356$, $P = 0.722$; Table 1). However, the number of orchid species per plot in the travertine area (3.23) was significantly greater than that in the forest area (2.37) ($t = 6.392$, $P < 0.001$; Table 1; Fig. 2). Most plots had two to five orchid species; the maximum number of orchid species per plot was nine occurring in one plot in the travertine area (Table 1; Fig. 2).

Frequency, cover and IPI of orchid species

Most orchid species (73.33% and 71.43% in the travertine and forest areas, respectively) had a frequency of less than 10% (Table 3). There were 18 and four orchid species, in the travertine and forest areas respectively, that had a frequency of less than 1% (Table 3). Six orchid species (*A. faberi*, *Coeloglossum viride*, *G. roborowskii*, *Gymnadenia orchidis*, *H. monorchis*, and *Platanthera fuscescens*) were each found only in single plots.

In the travertine area, the most frequent orchid species found were *C. flavum* (frequency 57.58%). *P. delavayi* (54.33%), *C. bardolphianum* (48.01%), *G. diantha* (43.14%), and *C. tibeticum* (40.43%), whereas in the forest area the most frequently occurring species were *T. szechuanica* (70.37%) and *Goodyera repens* (63.89%) (Table 3).

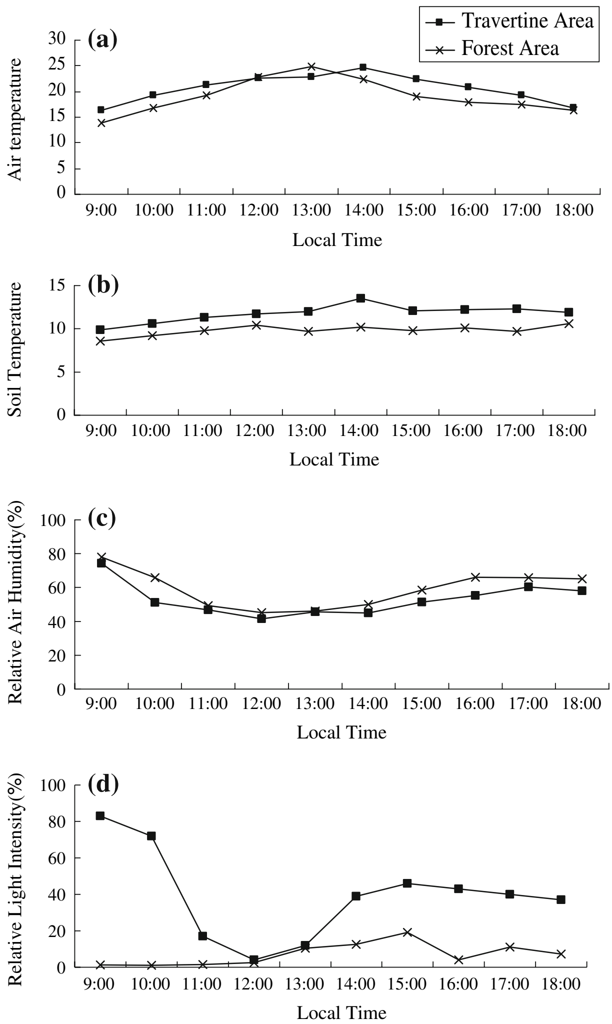

Fig. 1 Diurnal course of air temperature (°C) (**a**), soil temperature (°C) (**b**), relative air humidity (%) (**c**) and relative light intensity (%) (**d**)

The maximum cover values were 89% for *C. flavum*, followed by 76% for *P. delavayi* in travertine area (Table 3), which was much higher than the values for these two orchid species in forest (18% and 9% respectively). In comparison, the maximum cover value was 15% for the most frequent orchid species, *T. szechuanica* in the forest habitat, followed by *Goodyera repens* (9%) (Table 3).

In the travertine area, *C. flavum* had the highest IPI (16.94%), followed by *P. delavayi* (14.70%), *C. bardolphianum* (9.23%), *G. diantha* (8.37%), *C. tibeticum* (7.73%), and *Ponerorchis chusua* (5.87%) (Table 3). In contrast, orchid species having the highest IPI in the forest area were *T. szechuanica* (18.61%) followed by *Goodyera repens* (10.23%) (Table 3).

Table 2 Mean (±SE) values of properties of soils collected within travertine and forest areas

Soil property	Travertine area	Forest area
Moisture (%)*	36.67 ± 2.93	45.71 ± 2.29
Organic matter (%)**	4.08 ± 0.47	6.13 ± 0.35
Total nitrogen (%)**	0.63 ± 0.06	0.92 ± 0.07
Total phosphorus (mg/kg)^{NS}	965.81 ± 119.43	977.21 ± 49.06
Total potassium (mg/kg)^{NS}	3,051.38 ± 298.10	3,067.19 ± 254.92
Total calcium (mg/kg)**	150,773.66 ± 13,896.20	75,505.04 ± 15,671.81
pH***	7.50 ± 0.03	6.03 ± 0.24

Soil properties that were significantly different between travertine and forest are indicated with asterisks (*t*-test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Ns = Not significant). $N = 30$ within travertine and in forest areas respectively

Correlation between density and biotic conditions

The relationships between the densities of eight orchid species (*C. bardolphianum*, *C. flavum*, *C. tibeticum*, *Galearis diantha*, *Goodyera repens*, *Phaius delavayi*, *Ponerorchis chusua*, and *Tipularia szechuanica*) and biotic conditions were complex. In forest area, seven orchid species (no *C. tibeticum* distributed in forest area) showed no significant relationships with the percent coverage of tree, shrub, herb, moss, and soil depth except for *P. delavayi* which had a significant negative relationship with tree cover and *T. szechuanica* which had a significant positive relationship with soil depth (Table 4). In the travertine area, the densities of *C. bardolphianum*, *C. tibeticum*, and *Ponerorchis chusua* were negatively correlated with tree cover while *C. flavum*, *G. diantha*, and *P. delavayi* were positively correlated with tree cover (Table 4). The densities of the two common orchid species *Goodyera repens* ($P = 0.065$, and $P = 0.063$ for the travertine area and forest area respectively) and *T. szechuanica* ($P = 0.059$, and $P = 0.078$ for the travertine area and forest area respectively) in the forest had marginal positive relationship with tree cover.

Discussion

We have demonstrated that the Huanglong valley is rich in orchid species: a total 33 species of 21 genera were recorded in the study area, and have confirmed that the Huanglong valley is one of the most diverse sites for terrestrial orchids in China.

We observed that the travertine area and the forest area of the Huanglong valley differ greatly in orchid diversity and environmental conditions. The travertine area had higher numbers of orchid species as well as higher numbers of orchid species per plot than in the forest. We presume that the difference in tree cover and light availability between the two habitats might be responsible for these differences since a number of studies have reported that light is one of the factors limiting reproduction and colonization of some forest herbs (Chazdon and Fetcher 1984; Lubbers and Christensen 1986; Clark and Clark 1987; Hughes et al. 1988; Niesenbaum 1993; Cunningham 1997; Rankin and Tramer 2002) as well as for some orchid species (Kull 1998; Zhang et al. 2005). Effective acquisition and use of light is crucial, especially for plants occurring in the forest understory, as shading may not be favorable to growth and reproduction (Hughes et al. 1988), and thus the availability of light may be a determining factor influencing the fruit set, seedling survival, and growth rate of an

Table 3 Frequency, cover and important values of orchid species in the travertine areas ($N = 554$) and the forest area ($N = 108$) in Huanglong valley

Species	Travertine area				Forest area			
	Frequency (%)	Cover (%)		IPI(%)	Frequency (%)	Cover (%)		IPI (%)
		Average	Max			Average	Max	
Amitostigma faberi	0.18	0.0004	0.2	0.02	0	0	0	0
Calypso bulbosa	0.54	0.0014	0.5	0.07	17.59	0.0998	1.6	2.58
Coeloglossum viride	0.18	0.0009	0.5	0.02	0	0	0	0
Corallorhiza trifida	2.89	0.0286	15	0.40	3.7	0.0016	0.1	0.48
*Cypripedium bardolphianum****	48.01	2.2231	45	9.23	4.63	0.4037	12.6	1.92
*Cypripedium flavum****	57.58	6.7607	89	16.94	1.85	0.1704	18	0.80
Cypripedium guttatum	0.90	0.0107	4	0.13	0	0	0	0
Cypripedium calcicolum	0.54	0.0162	5.6	0.09	0	0	0	0
Cypripedium tibeticum	40.43	1.8403	30.6	7.73	0	0	0	0
Didiciea cunninghami	0.36	0.0038	1.5	0.06	4.63	0.0509	1.8	0.76
Epipogium aphyllum	0.18	0.0007	0.4	0.02	0.93	0.0011	0.12	0.12
Epipactis helleborine	0.90	0.0078	1.2	0.12	0	0	0	0
*Galearis diantha****	43.14	2.0475	45	8.37	5.56	0.0301	2	0.81
Galearis roborowskii	0.18	0.0009	0.5	0.02	0	0	0	0
Galearis sp.	0.54	0.0188	5.7	0.10	0	0	0	0
*Goodyera repens****	12.82	0.0494	2.1	1.69	63.89	0.6251	7.5	10.23
Goodyera wolongensis	0	0	0	0	7.41	0.0292	0.9	1.05
Gymnadenia orchidis	0.18	0.0009	0.5	0.02	0	0	0	0
Herminium monorchis	0.18	0.0032	1.8	0.02	0	0	0	0
Listera biflora	0	0	0	0	2.78	0.0042	0.2	0.36
Listera puberula var. *maculata*	0.54	0.0199	6	0.10	0.93	0.0083	0.9	0.15
Listera smithii	0.72	0.0224	6	0.12	15.74	0.0481	0.8	2.17
Malaxis monophyllos	4.33	0.8887	75	1.82	6.48	0.1815	6.8	1.43
Neottia acuminate	0.18	0	0.02	0.02	10.19	0.0096	0.2	1.33
Neottia listeroides	0.36	0.0006	0.25	0.05	1.85	0.0037	0.3	0.25
Neottianthe monophylla	0.90	0.0153	4	0.13	0	0	0	0
Oreorchis nana	6.68	0.1078	11.6	0.99	0.93	0.025	2.7	0.20
*Phaius delavayi****	54.33	5.4872	76	14.70	1.85	0.1111	9	0.61
Platanthera fuscescens	0	0	0	0	0.93	0.0167	1.8	0.18
Platanthera minor	0.36	0.011	4.5	0.07	0	0	0	0
Platanthera minutiflora	4.51	0.0372	2.4	0.62	13.89	0.1352	4.3	2.22
*Ponerorchis chusua****	37.00	0.8419	40	5.87	1.85	0.0157	1.4	0.29
*Tipularia szechuanica****	2.35	0.0568	15	0.38	70.37	2.9094	15	18.61

Important value index (IPI) = (Relative Frequency (%) + Relative cover (%))/2. Frequency that was significantly different between travertine and forest areas are indicated with asterisks (Chi-Square Test, for *Cypripedium bardolphianum*, *C. flavum*, *C. tibeticum*, *Galearis diantha*, *Goodyera repens*, *Phaius delavayi*, *Ponerorchis chusua*, and *Tipularia szechuanica* only). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Ns = Not significant

orchid population (Mckendrick 1996; Willems et al. 2001; Janečková et al. 2006). The percent cover of overstory is a critical factor that determines the quality and quantity of light reaching the understory plants (Cunningham 1997; Le Brocque and Buckney 2003; Zhang et al. 2005). The more open habitat of the travertine, with its sparse tree cover, would allow more light to reach the understory relative to the forest area (Table 1b; Fig. 1d), and thus there could be less competition among the understory plants for light (Shrestha et al. 2000). Eventually, more orchid species could become resident in the travertine area than in the

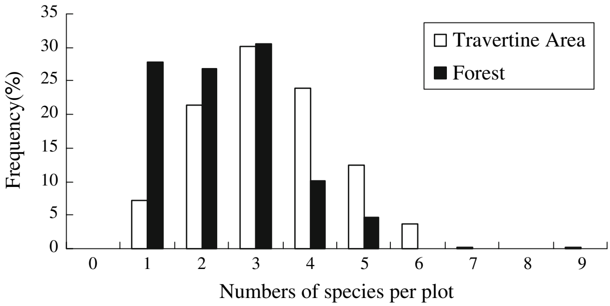

Fig. 2 Frequency distribution of orchid species of plots sampled in travertine area ($N = 554$) and in forest area ($N = 108$) in Huanglong valley. Most species occurred in less than 10% of plots (73.33% and 71.43% for travertine area and forest area respectively). Most plots contained 2–5 orchid species in travertine area and 1–3 orchid species in forest

forest area (Behera and Misramk 2006). The distribution pattern of orchid species could be a reflection of adaptation to a certain light environment (Killingbeck et al. 1998). The orchid species *C. bardolphianum*, *C. tibeticum*, and *Ponerorchis chusua* in the travertine area are negatively correlated with the percentage cover of trees (Table 4) and have much lower frequency in the shady forest area (Table 3), indicating that those orchid species appear to favor high light microenvironments, whereas the most common orchid species *T. szechuanica* and *Goodyera repens* in forest area were marginally positively correlated with the percentage tree cover and have lower frequency in travertine area, indicating that those orchid species may have lower compensation points.

The diurnal course of air temperature, soil temperature and relative humidity were significantly different between the two habitats (*t*-test: all $P < 0.016$; Fig. 1a–c), these differences or combinations of these differences were likely to have played a role in determining the distribution of orchid species (Brzeskiewicz 2000; Maestre 2004). In this study, despite we monitored the diurnal course of air temperature, soil temperature, relative air humidity and relative light intensity only at one point for one day, we considered that those data provides a rough estimate of the differences in the microenvironmental conditions between the two habitats. Obviously, it is necessary to gather more data in future to assess the influence of air temperature, soil temperature, relative air humidity and relative light intensity on the distribution of orchid species in two habitats.

Stream flows through the travertine area might also play an important role in influencing orchid richness of the Huanglong valley. The stream flows through the travertine area from April to November every year which is consistent with the growing season of orchids. The stream flows may be crucial in shaping and maintaining the stable microenvironment for the orchid populations to persist (Jones 1998) particularly since some non-orchidaceous species that cannot tolerate the high calcium content of the water would likely be out-competed in the travertine community (Stuckey 1967; Perner and Luo 2007), and thus reduce the competition for light, which favors orchid growth and reproduction (Janečková et al. 2006). We have assumed that stream flow could be considered a disturbance factor because after rain, the stream flow often spreads over the vegetated patches of the travertine area (Lytle and Poff 2004). A number of studies have shown that moderate disturbance can reduce the competition of shrubs and herbs which in turn favors orchid species (Primack et al. 1994; Catling 1996; Jones 1998; Killingbeck et al. 1998; Wheeler et al. 1998; Kull 1999; Tali et al. 2004; Coates et al. 2006; Janečková et al. 2006) but suitable disturbance could also provide a greater opportunity for herbaceous species to invade (Mishra et al. 2004; Behera and

Table 4 Spearman rank correlation coefficients (r) between density (plants/plot) of eight orchids and percent coverage of tree, shrub, herb, moss, and soil thickness in the travertine areas ($N = 554$) and the forest area ($N = 108$) in Huanglong valley

Density of orchid species	Travertine area					Forest area				
	Tree cover	Shrub cover	Herb cover	Moss cover	Soil depth	Tree cover	Shrub cover	Herb cover	Moss cover	Soil depth
Cypripedium bardolphianum	−0.125**	0.102*	0.014^{Ns}	-0.028^{Ns}	-0.011^{Ns}	0.007^{Ns}	0.073^{Ns}	0.116^{Ns}	0.104^{Ns}	-0.165^{Ns}
Cypripedium flavum	0.115**	−0.088*	0.162**	0.003^{Ns}	0.244**	0.044^{Ns}	-0.093^{Ns}	-0.018^{Ns}	0.065^{Ns}	0.007^{Ns}
Cypripedium tibeticum	−0.192**	0.060^{Ns}	-0.031^{Ns}	−0.164**	-0.081^{Ns}	–	–	–	–	–
Galearis diantha	0.090*	-0.053^{Ns}	0.157**	0.118**	−0.107*	0.086^{Ns}	0.031^{Ns}	0.153^{Ns}	0.115^{Ns}	0.090^{Ns}
Goodyera repens	0.065^{Ns}	0.110**	−0.203**	0.078^{Ns}	0.156**	0.179^{Ns}	-0.152^{Ns}	-0.111^{Ns}	-0.076^{Ns}	0.064^{Ns}
Phaius delavayi	0.129**	-0.043^{Ns}	0.084*	0.055^{Ns}	0.221**	−0.210*	0.073^{Ns}	-0.018^{Ns}	0.065^{Ns}	0.022^{Ns}
Ponerorchis chusua	−0.090*	-0.018^{Ns}	0.097*	−0.172**	−0.224**	-0.119^{Ns}	0.009^{Ns}	0.054^{Ns}	0.065^{Ns}	-0.159^{Ns}
Tipularia szechuanica	0.059^{Ns}	-0.047^{Ns}	−0.085*	0.044^{Ns}	0.091*	0.078^{Ns}	0.011^{Ns}	0.040^{Ns}	0.056^{Ns}	0.200*

Ns = no significant correlation ($P > 0.05$); *$P < 0.05$, **$P < 0.01$. "–" means no data were available

Misramk 2006; Janečková et al. 2006). Furthermore, opening of the overstory favors orchid invasion (Devi and Behera 2003), and therefore could be responsible for the higher number of orchid species seen in the travertine area than in the forest area.

The high numbers of orchid species were found in both habitats, but most species had a frequency of less than 10% (Table 3). In particular, 18 orchid species were present in less than 1% of the plots in the travertine area and this was 4.5 times more than that seen in the forest (Table 3). This may be because there are more new habitats to be invaded in the travertine area due to the water disturbance and high light availability, and those new habitats would have low numbers of orchid individuals and therefore result in higher number of orchid species with comparatively lower frequency in that area.

Mycorrhizal fungi are considered necessary for seed germination and growth of terrestrial orchids (Arditti et al. 1990; Rasmussen and Whigham 1998; Batty et al. 2001). However, the composition and distribution of mycorrhizal fungi could be affected by biotic factors, disturbance (e.g. frequent fires), and soil factors or combinations of these factors, which in turn may determine recruitment success and ultimately the distribution of orchid plants in the field (Stuckey 1967; Perkins et al. 1995; Arntz 1999; Batty et al. 2000; Brundrett et al. 2003). Brundrett et al. (2003) demonstrated that most fungal activity occurred in coarse organic matter in the topsoil and litter layers, which resulted in higher orchid seed germination rates because of higher densities of mycorrhizal fungi and more nutrients to feed orchid fungi. The soil depth, thus, could be considered having no strong effect on orchid seed germination. The differences of the cover of trees, shrubs, herbs, and mosses between travertine and forest areas could lead to differences in the litter layer which could influence mycorrhizal fungi composition and activity which in turn could influence orchid seed germination. Our results indicated that the organic matter was much higher in the forest area (mean = 6%, travertine = 4%; Table 2). Batty et al. (2001) reported that seed germination rate in *Caladenia arenicola* had a negative relationship with the level of organic carbon. This may be because organic matter is likely related to mycorrhizal fungi, soil structure and texture properties, and may impose both direct and indirect impact on seed germination and thus the orchid distribution patterns (Batty et al. 2001; Brundrett et al. 2003). Therefore, the cover of trees, shrubs, herbs, and mosses as well as organic matter may play a role in determining the orchid species composition in the two habitats.

The calcium content of soil was extremely high in both areas (Table 2). It is believed that certain orchid species prefer calcareous soil (Hull and Ring 1995; Bournérias 1998; Kull 1999; Whigham and Willems 2003). Bournérias (1998) reported that the greatest orchid diversity occurs in limestone areas of Europe. Ninety percent of European terrestrial orchid species are distributed in Mediterranean (Whigham and Willems 2003), especially to the east of the Mediterranean, where the diversity of orchid species is extremely high including many endemic orchid species on islands with calcareous soil (Whigham and Willems 2003). Thus, we suggest that the calcareous soil of the Huanglong valley site may play a role in supporting the high orchid species diversity occurring in this area. However, we have yet to learn why orchid species prefer calcareous soil. Tremblay et al. (1998) suggested that some plants may be unsuited to calcareous soil and/or that calcareous soils may be particularly favorable to the symbiotic fungi which play an extremely important role in orchid species life history.

Conservation implications

The microenvironmental conditions and the orchid species compositions were found to be quite different in the travertine and forest areas. These differences may be the result of changed environmental conditions which followed the historic interrupted stream flow. Studies have shown that altered stream flow regimes can strongly impact species composition and vegetation structure (Stromberg et al. 2007). We assume that community succession occurred in the area where stream flow had ceased. The habitat gradually became unfavorable for some orchid species because of increasing overstory and competition with non-orchidaceous species for light. Therefore, the pattern and composition of orchid species distribution which we see today is the results of the process of habitat changes. Our study suggests that some orchid species in forest are faced with an uncertain future, that the numbers of them will continue to decline and perhaps even disappear from the site except for the very few that possess the ability to adapt to the changing environment. This seems to be supported by some data, for example, the frequency and cover of orchid species *C. bardolphianum*, *C. flavum*, *C. tibeticum*, *G. diantha*, *P. delavayi* and *Ponerorchis chusua* were considerably lower in forest as compared with the travertine area. Moreover, nearly all the orchid species found in Huanglong valley occur in the travertine area while the adjacent forest area has few orchids except the particular patch mentioned in the introduction. It is suggested that some forest species may be declining yet some species were only recorded in the forest (Table 3). An alternative suggestion would simply be that some species occur at naturally lower levels in forested habitats or that environmental conditions in the forest may be unfavorable to persistence of other species. Environmental conditions may determine fitness to a greater degree than do plant traits themselves (Herrera 1993; O'Connell and Johnston 1998). The results presented in this study imply that it could be a calamity for some orchid species if the travertine stream flow was changed or diminished.

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