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Impact of proximity to a pathway on orchid pollination success in Huanglong National Park, South-West China

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ABSTRACT

Although edge effects often significantly alter abiotic habitat variables, and the performance and behaviour of plant and animal species, they are rarely taken into account when designing nature reserves or managing the movement of visitors within reserves. In this study, we examined the effects of proximity to a habitat edge, created by a visitor pathway, on reproductive success of the orchids *Phaius delavayi* and *Ponerorchis chusua* within the Huanglong National Park, China. Parallel transects were established at different distances from the pathway to test the effects of proximity to the pathway on male and female reproductive success in two consecutive years. For *Phaius delavayi*, percentage pollinarium removal and percentage fruit set showed significant variation between transects in both years, with plants in transects closer to the pathway having lower reproductive success. *P. chusua* showed the same trends except for pollinarium removal in one year. These results are consistent with the hypothesis that proximity to the pathway, which is used by a large number of visitors every day throughout the orchids' flowering seasons, interfered with pollinator activity, depressing male and female reproductive success in both species. The impacts on reproductive success were significant for plants within approximately 10 meters of the pathway. Such edge effects may be common, and may need to be considered when designing nature reserves in which visitor activity is encouraged.

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1. Introduction

Fragmentation of a habitat increases the proportion of its remnant that is potentially influenced by edge effects (Murcia, 1995; Weiermans and van Aarde, 2003). Edge effects occur

close to the boundary between two different habitat types delineated by an abrupt edge. Such abrupt edges are often created anthropogenically (Murcia, 1995; Foggo et al., 2001). Edge effects include alterations in the performance and behaviour of plant and animal species, often as a result of

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changes in abiotic conditions (Lienert and Fischer, 2003). Most edge effects decline in magnitude with distance from the habitat edge, and cease to be significant within 50 m (Murcia, 1995).

One important way in which conditions at the edges of habitats can affect plants is by changing the behaviour of pollinators (Aguilar et al., 2006). Many pollinators are adversely affected by conditions near the edges of habitat fragments, declining in abundance (Fowler et al., 1993; Liu and Koptur, 2003) and altering their behaviour and frequency of flower visitation (Didham et al., 1996; Aguilar et al., 2006). Such changes might be expected to reduce pollination efficiency, resulting in lower fruit production and seed set (Fagan et al., 1999; Honnay et al., 2005; Valdivia et al., 2006). Despite their potential importance for the relationships between plants and their pollinators, however, few studies have measured the impact of habitat edges on plant reproductive success (Chacoff and Aizen, 2006; Ewers and Didham, 2006). Information about the impacts of habitat edges on reproductive success would be valuable in designing nature reserves in which rare plants are managed for their conservation, and visitation by the public is also encouraged (Sugiura et al., 2001). Nature reserves are increasingly being in the second of these roles in many parts of the world because of public interest, for education and ecotourism, and because revenue can be generated from such activities. Understanding how visitor facilities, and the presence of visitors, affect species within reserves will allow designs to be refined and patterns of usage modified to minimize adverse effects.

The reproductive success of obligately animal-pollinated plants is likely to be especially sensitive to a decrease in pollinator visitation because of reduced pollen transport and deposition (Cresswell, 1997; Aguilar and Galetto, 2004; Pemberton and Liu, 2008). In this paper we examine the impact of proximity to a habitat edge on pollinium export and pollinium deposition in two terrestrial orchid species, *Phaius delavayi* (Finet) P.J. Cribb et Perner and *Ponerorchis chusua* (D. Don) Soó. As in many species in the Orchidaceae, pollination success in these species is strictly dependent on visitation by pollinium vectors (Pellegrino et al., 2005; Tremblay et al., 2005). The species share the same pollinators (*Bombus hypnorum* and *B. lepidus*, Hymenoptera, Li Peng, personal communication), and are deceptive orchids, i.e. they do not reward their pollinators (Schiestl, 2005). We examined percentage pollinarium removal and percentage fruit set in both species within a set of parallel transects located different distances from a visitor pathway constructed through the orchid habitat. Our hypothesis was that, if edge effects have a negative impact on the relationship between the orchid species and their pollinators, this would be reflected in increasing male and female reproductive success, estimated respectively as pollinarium removal and fruit set, with distance from the pathway.

2. Materials and methods

2.1. Study site

The study was conducted in Huanglong Valley within Huanglong National Park, a Natural World Heritage Site (32°41'–

32°54'N, 103°44'–104°3'E) in Sichuan, southwestern China. Mean annual temperature and mean annual precipitation are 5 °C and 760 mm respectively, and most rainfall (70–73% of annual precipitation) occurs between May and September (Ran and Liu, 2002). The valley is 3.5 km long and up to 300 m wide at altitudes of between 3100 and 3570 m asl (Ran and Liu, 2002). A visitor pathway 3 m wide has been constructed within the valley. It is used from 07.00 to 18.00 h, with the peak time for visitors being between 11.00 and 15.00 h every day from May to October. Over 1.5 million visitors are attracted to the site per year.

2.2. Study species

P. delavayi (Finet) P.J. Cribb et Perner is a long-lived, polycarpic, pseudobulbous clonal orchid species which is endemic to China (Tsi, 1999). The inflorescence is sparsely 1–7-flowered (usually 3–5 flowers). Flowers are yellow with red markings on the labellum. No floral nectar or other form of pollinator reward is produced. There is one anther with eight pollinia within each flower. The inflorescence is up to 30 cm in height, and the flowering period extends from mid-June to late-July in the study population. The longevity of individual flowers is approximately 25 days.

P. chusua (D. Don) Soó. is a non-clonal orchid with one or two underground tubers. It produces an inflorescence with 1–20 (usually 2–9) flowers in the study area. Flowers are small, purple or pink, with black spots on the trifold labellum, and they lack nectar. There is one anther with two pollinia per flower. The inflorescence is up to 25 cm in height, and the flowering period in the study population is early to late-July. The longevity of individual flowers is approximately 20 days.

In Huanglong valley, *P. delavayi* and *P. chusua* grow where there is sufficient light and moisture, in meadows and open scrub with occasional trees including *Abies faxoniana*, *A. ernestii* and *Betula utilis*, and shrubs including *Dasiphora fruticosa*, *Salix tetrasperma*, *Berberis polyantha*, *Sorbus hupehensis*, *Lonicera* sp., and *Ribes* sp. Both species are found growing with many other orchid species such as *Cypripedium bardolphianum*, *C. flavum*, *C. tibeticum*, *Oreorchis nana*, *Galearis diantha*, and non-orchidaceous species including *Polygonum sphaerostachyum*, *Allium prattii*, *Arctous ruber*, *Gentiana scabra*, *Elymus nutans* and *Carex lehmanii* (Huang et al., 2007).

2.3. Methods

Field work was conducted during the growing season (between May and September) in 2006 and 2007. For *P. delavayi*, data were collected on plants located within five adjacent transects, each 5 m wide and 50 m long, oriented parallel to the pathway. The boundaries of the five transects were 0–5 m, 5–10 m, 10–15 m, 15–20 m, and 20–25 m from the pathway. The transects were numbered 1 (the nearest to the pathway) to 5 (the furthest from the pathway). In each of the 2 years, 35 flowering plants of *P. delavayi* were selected at random from within each of the five transects for measurements. Because of the uneven distribution pattern of *P. chusua* throughout the study area, only two of the transects (those with boundaries 0–5 m and 10–15 m from the pathway, i.e. transects 1 and 3) were sampled for this species. In each of

the two years, thirty flowering individuals of *P. chusua* were selected at random for measurements from within each of these two transects. Different plants of each species were measured in each year of the study.

All selected plants were marked. For each plant, the number of flowers in each inflorescence, inflorescence height (vertical distance from ground to the top of the inflorescence), and the distance to the nearest flowering conspecific neighbour (the last trait only for *P. delavayi*) were measured.

Due to very low pollinator visitation frequency in many orchid species, pollinarium removal can be used both as an indirect reflection of relative pollinator abundance and visitation frequency, and as a measure of male reproductive success (Broyles and Wyatt, 1990; Fritz and Nilsson, 1996; Vallius and Salonen, 2000; Parra-Tabla and Vargas, 2004). It can also give a more reliable indication of the frequency and efficiency of visiting pollinators than that obtained from measurement of fruit set (Broyles and Wyatt, 1990; Fritz and Nilsson, 1996). The absence of the pollinarium was therefore also recorded for every flower. We recorded pollinarium removal in every flower in each of the inflorescences of selected plants every three days during the flowering period. Male reproductive success was calculated for each individual plant as (the cumulative number of flowers with pollinarium removed over the flowering season/total number of flowers in the inflorescence) \times 100%. In late September, we counted the number of fruits produced by each plant, and female reproductive success was calculated as (number of fruits produced/number of flowers in the inflorescence) \times 100%. We also calculated mean (\pm SE)% reproductive success for all of the orchids recorded within each of the transects for both species.

In 2006, five additional flowering individuals of *P. delavayi* were randomly selected for hand pollination within each of the five transects. Every flower in each of the inflorescences was artificially pollinated using pollinaria from conspecific flowers located at least 10 m away from each of the selected plants. Fruits were counted on these plants in late September, and their female reproductive success calculated as above.

2.4. Pollinator observation

In 2006, observations were made on visits by pollinating insects to flowering *P. delavayi* plants within each of the five transects. Data were collected between 09.00 and 17.00 h from 28 June to 15 July, on all sunny days when weather conditions favoured pollinator activity. Each period of observation lasted for 30 min. To avoid possible bias caused by time-dependent pollinator activity (Mustajärvi et al., 2001), the time for observation periods within each transect was selected randomly between 09.00 and 17.00 h. In total, there were 24 observation periods, each of 30 min, for each of the five transects (12 h per transect, and 60 h in total).

2.5. Data analysis

Analysis of covariance (ANCOVA) was used to examine the impact of distance from the pathway on male and female reproductive success in both species, with percentage pollina-

rium removal and percentage fruit set of plants in different transects as dependent variables, transect as a fixed factor, and inflorescence height and distance to nearest flowering conspecific neighbour (see below) as covariates. When results were significant, Bonferroni pair-wise post-hoc tests were used to determine which mean values were significantly different between transects. Since different plants were sampled in each year of the study, separate analyses were conducted for each year. Correlations between variables were examined using Spearman Rank Correlations. Mean values of number of flowers per inflorescence, height of inflorescence and distance to nearest flowering conspecific were compared within transects between years and between transects within years, by ANOVA. SPSS Version 11.5 for Windows was used for all tests.

3. Results

3.1. Plant traits

Mean number of flowers per inflorescence, inflorescence height, and mean distance to nearest flowering conspecific neighbour (for *P. delavayi* only) are shown in Table 1 for plants recorded within each transect in both years. Within each of the five transects there were no significant differences between years in any of the trait values ($P > 0.05$ for all traits, using ANOVA). Thus there was no evidence of changes in quality of habitat between years, as measured by plant performance at different distances from the pathway. However, for both orchids, and in both years, there were significant differences in several trait values between transects. For example, for *P. delavayi*, inflorescence height showed significant variation between transects ($P < 0.001$ in both 2006 and 2007, Table 1).

Correlations between plant traits, based on all measurements taken from plants in all transects in 2006, are presented in Table 2. There were strong correlations between the number of flowers in inflorescences and inflorescence height in both species (Spearman rank correlation, $P < 0.001$ for both species, Table 2). Percentage pollinarium removal and percentage fruit set showed stronger correlations with inflorescence height than with number of flowers in the inflorescences in *P. delavayi* (Table 2). Inflorescence height was not correlated with distance to nearest flowering conspecific neighbour. Consequently we used inflorescence height and distance to nearest flowering conspecific neighbour (this trait for *P. delavayi* only), as covariates in the following analyses.

3.2. Male reproductive success

Phaius delavayi: Mean percentage pollinarium removal showed significant variation between the five transects in both years of the study (ANCOVA, $P < 0.044$ in both years, Table 3), and was lowest in transect 1 (i.e. the transect 0–5 m from the pathway) in both years. In 2006, male reproductive success was highest in transect five (Fig. 1), and nearly 2.6 times greater in this transect than in transect 1. Mean percentage male reproductive success increased with distance of transect from the pathway in both years of the study (Fig. 1).

Table 1 – Mean (\pm SE) number of flowers per inflorescence (FN), inflorescence height (INFH), and distance to the nearest flowering conspecific neighbour (NND) for *Phaius delavayi* in five transects and *Ponerorchis chusua* in two transects in 2 years in Huanglong Valley. Transect 1 was closest to the pathway and transect five furthest away. “–” = Not measured. Different superscript letters within each column indicate significant differences ($p < 0.05$).

	2006			2007		
	FN	INFH (cm)	NND (cm)	FN	INFH (cm)	NND (cm)
<i>Phaius delavayi</i>						
Transect 1	2.5 \pm 0.2 ^a	18.5 \pm 0.5 ^a	9.6 \pm 0.9 ^a	2.8 \pm 0.2 ^a	16.3 \pm 0.7 ^a	10.1 \pm 1.3 ^b
Transect 2	3.0 \pm 0.1 ^{ab}	22.1 \pm 0.5 ^b	7.4 \pm 0.7 ^a	3.3 \pm 0.1 ^b	20.5 \pm 0.5 ^b	7.6 \pm 0.7 ^{ac}
Transect 3	3.1 \pm 0.1 ^{ab}	22.0 \pm 0.4 ^b	5.5 \pm 0.5 ^b	3.3 \pm 0.2 ^b	19.1 \pm 0.5 ^{bd}	6.1 \pm 0.5 ^{ad}
Transect 4	2.6 \pm 0.0 ^{ab}	18.8 \pm 0.4 ^a	6.6 \pm 0.8 ^b	3.8 \pm 0.1 ^{ce}	20.4 \pm 0.7 ^b	4.9 \pm 0.5 ^a
Transect 5	3.5 \pm 0.2 ^c	20.9 \pm 0.9 ^{ab}	6.9 \pm 0.8 ^a	3.8 \pm 0.2 ^{de}	16.6 \pm 0.8 ^{cd}	8.1 \pm 0.7 ^{bcd}
<i>Ponerorchis chusua</i>						
Transect 1	4.8 \pm 0.3 ^a	17.0 \pm 0.6 ^a	–	5.1 \pm 0.3 ^a	17.7 \pm 0.7 ^a	–
Transect 3	3.4 \pm 0.2 ^b	11.2 \pm 0.49 ^b	–	4.1 \pm 0.3 ^b	11.3 \pm 0.4 ^b	–

Table 2 – Spearman’s rank correlation coefficients between measured plant traits in *Phaius delavayi* and *Ponerorchis chusua* in 2006 in Huanglong Valley. “–” = Not measured.

	Flower number	Inflorescence height	Distance to nearest neighbour	Pollinarium removal
<i>Phaius delavayi</i>				
Inflorescence height	0.610 ^{***}			
Distance to the nearest neighbour	0.002 ^{ns}	–0.110 ^{ns}		
Pollinarium removal	0.217 ^{**}	0.245 ^{***}	0.082 ^{ns}	
Fruit set	0.082 ^{ns}	0.161 [*]	0.076 ^{ns}	0.617 ^{***}
<i>Ponerorchis chusua</i>				
Inflorescence height	0.736 ^{***}			
Pollinarium removal	–0.064 ^{ns}	–0.058 ^{ns}	–	
Fruit set	0.053 ^{ns}	–0.072 ^{ns}	–	0.702 ^{***}

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

ns Not significant.

P. chusua: Mean percentage pollinarium removal was significantly lower closer to the pathway in 2006 (ANCOVA, $P = 0.013$, Table 3, Fig. 1), but there was no significant difference in mean percentage pollinarium removal between the two transects in 2007 (ANCOVA, $P = 0.892$, Table 3, Fig. 1).

3.3. Female reproductive success

Phaius delavayi: All artificially pollinated flowers of *P. delavayi* developed mature capsules in all five transects in 2006. Thus, fruit set was 100% when all flowers in an inflorescence were hand-pollinated.

Just as for male reproductive success, there was a significant effect of distance from the pathway on percentage female reproductive success in *P. delavayi* in both years (ANCOVA, $P < 0.036$ in both years, Table 3). In 2006, mean percentage fruit set in the transect furthest from the pathway was 2.2 times greater than in the transect closest to the pathway (Fig. 1). In both years of the study there was an increase in female reproductive success with distance from the pathway (Fig. 1).

P. chusua: There were significant differences in percentage fruit set between the two transects in both years (ANCOVA,

$P < 0.038$ in both years). Fruit set was 1.8 and 1.4 times higher further from the pathway in 2006 and 2007, respectively (Fig. 1).

3.4. Pollinator observation

Over the whole period of observation, only one visit by a pollinating species (*Bombus hypnorum*) to the study species was recorded. During this foraging bout, 12 flowers from seven different plants of *P. delavayi* were visited. All plants visited were within the transect furthest from the pathway. Because pollinator observation was so unproductive, we did not attempt to collect further data on visitation frequency in 2007, and were unable to undertake statistical comparisons of visitation frequency to plants in different transects.

4. Discussion

For both orchid species, in both years of this study, proximity to the pathway significantly affected both male and female reproductive success, with both increasing with distance from the pathway. Proximity of conspecific flower-

Table 3 – ANCOVA for pollinarium removal and fruit set per inflorescence for *Phaius delavayi* in five transects and *Ponerorchis chusua* in two transects in Huanglong Valley. Transect as fixed factor and inflorescence length and (for *P. delavayi* only) distance to the nearest flowering conspecific neighbour as covariates.

Source of variation	2006				2007			
	Type III MS	d.f.	F	P	Type III MS	d.f.	F	P
<i>Phaius delavayi</i>								
Pollinarium removal								
Transect	28611.1	4	9.5	0.000	7230.2	4	2.5	0.044
Inflorescence height	14110.7	1	16.8	0.000	4384.9	1	6.1	0.015
Distance to nearest neighbour	8146.1	1	9.7	0.002	75.5	1	0.1	0.747
Error	141367.3	168			121322.7	168		
Fruit set								
Transect	19788.4	4	7.2	0.000	4778.6	4	2.6	0.036
Inflorescence height	7095.9	1	10.3	0.002	4421.3	1	9.7	0.002
Distance to nearest neighbour	6534.4	1	9.4	0.002	28.9	1	0.06	0.801
Error	116236.4	168			76211.2	168		
<i>Ponerorchis chusua</i>								
Pollinarium removal								
Transect	6914.3	1	6.6	0.013	19.3	1	0.02	0.892
Inflorescence height	2049.9	1	2.0	0.167	2532.6	1	2.4	0.124
Error	61652.3	57			59263.3	57		
Fruit set								
Transect	7162.1	1	6.1	0.012	4111.7	1	4.5	0.038
Inflorescence height	1888.9	1	1.8	0.187	1391.2	1	1.5	0.222
Error	62466.3	57			51950.8	57		

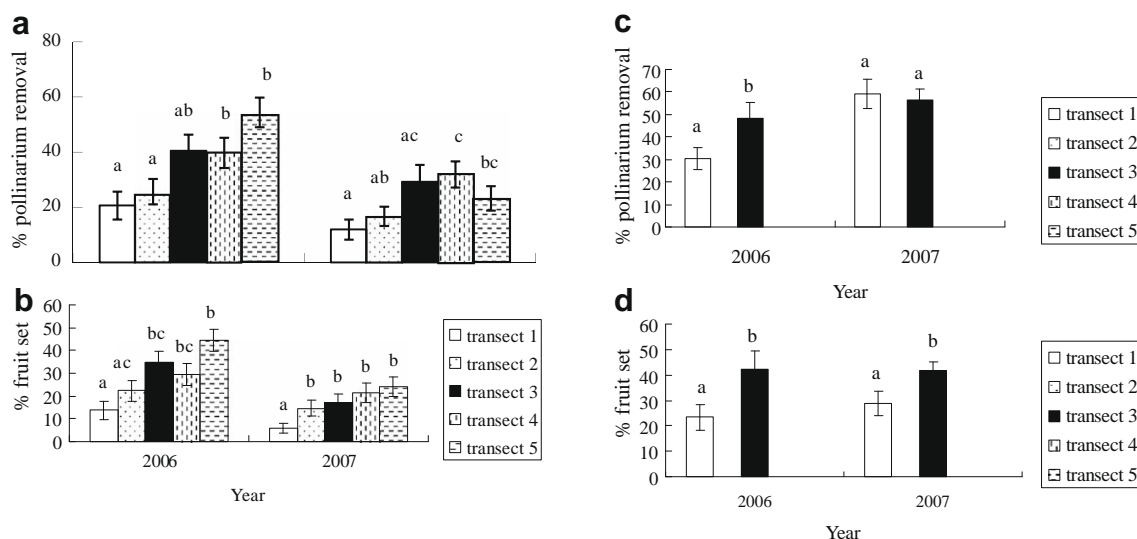


Fig. 1 – Mean (\pm SE) male (a) and female (b) reproductive success of *Phaius delavayi* in five transects and mean (\pm SE) male (c) and female (d) reproductive success of *Ponerorchis chusua* in two transects in Huanglong valley in 2006 and 2007. Transect 1 was the nearest to, and transect 5 the furthest away from, the pathway. Bars with different letters are significantly different ($P < 0.05$).

ing individuals can influence plant reproductive success (Roll et al., 1997; Meléndez-Ackerman and Ackerman, 2001; Wagenius, 2006). However, including distance to the nearest flowering conspecific neighbour as a covariate did not change the effect of distance of transect from the pathway on either male or female reproductive success in *P. delavayi* in either year (Table 3). The effect of distance of transect from the pathway on reproductive success was therefore

independent of distance to the nearest conspecific flowers in all transects. Moreover, few flowers of other species were open at the same time as those of the two species studied, and bumblebees seldom visited them (Huang, personal observation). Thus, simultaneously-flowering species are also unlikely to have strongly influenced pollinator visitation rates or the reproductive success of the orchid species in the study area.

Availability of resources is unlikely to have constrained female reproductive success in *P. delavayi*, at least in 2006, because all hand-pollinated flowers on all treated inflorescences in all transects produced mature capsules. This suggests that the differences in pollination success between transects in this study were caused by differential visitation frequency or efficiency of pollinators, rather than by differences in resource limitation between transects (Mattila and Kuitunen, 2000; Parra-Tabla et al., 2000; Vallius, 2001).

Inflorescence height showed significant variation between transects, and was significantly correlated with male and female reproductive success in *P. delavayi* (Table 2). However, the inclusion of inflorescence height as a covariate did not change the effect of transect position on either male or female reproductive success for either orchid species (Table 3). For *P. chusua*, mean number of flowers per inflorescence and mean inflorescence height were greater closer to the pathway in both years. Generally, taller inflorescences contain more flowers and are more attractive to pollinators (Tremblay et al., 2005), and therefore higher reproductive success might have been expected in *P. chusua* in the transect closer to the pathway. Given the absence of alternative explanations, and the strict dependence of reproductive success in both orchid species on pollinium vectors, it seems reasonable to conclude that the effects of distance from the pathway on male and female reproductive success are caused by differences in the frequency of pollinator visitation.

For *P. delavayi*, pollinarium removal and fruit set in the transect closest to the path were approximately 40% and 30% of the highest mean levels of male and female reproductive success seen in naturally-pollinated plants in 2006, and only 30% and 20% of the highest values in 2007. The highest values in both years were recorded in the transect furthest from the pathway, and there were more or less continuous increases in both male and female mean reproductive success with increasing distance from the path, suggesting a more or less monotonic increase in the intensity of the edge effect (Happer et al., 2005).

The way in which the habitat edge affects pollinators and pollination is unclear (Tscharntke et al., 2002; Jules and Shahani, 2003; Chacoff and Aizen, 2006). Anthropogenic barriers such as roads and railways can restrict bumblebee movement between parts of a sub-divided population (Bhattacharya et al., 2003). In the area where this study was carried out, more than ten thousand tourists walk along the pathway every day during the orchids' flowering season. We suggest that this affects the behaviour of bumblebees and adversely alters the interaction between them and the plants they pollinate (Sun and Walsh, 1998; Sugiura et al., 2001). Other studies have shown that human activity can have direct negative effects on plant species and communities (García et al., 2002; Kelly et al., 2003), by altering the local range and abundance of some plant species (Malo et al., 2001). Sun and Walsh (1998) and Sugiura et al. (2001) also claim that tourism can affect plants indirectly by altering the interactions between them and their pollinators. Pollinating insects have been shown to change their behaviour and avoid sites where humans are present, perhaps because anthropogenic disturbance is associated with predation risk (Steidl and Anthony, 2000; Gill et al., 2001; Frid and Dill, 2002), and *Bombus* spp. are strongly

affected by changes in environmental conditions (Parra-Tabla et al., 2000; Goverde et al., 2002; Bhattacharya et al., 2003). Animal-pollinated plants may also be highly susceptible to such edge effects (Cunningham, 2000; Aguilar and Galetto, 2004) as a consequence of reduced pollination efficiency (Jules and Rathcke, 1999; Goverde et al., 2002; Honnay et al., 2005), leading to lower fruit production or seed set (Jules and Rathcke, 1999; Robertson et al., 1999; Jacquemyn et al., 2002). Sugiura et al. (2001) found that fruit set of *Cypripedium macranthos* var. *rebunense* was lower at a site with many human visitors than at a site with none, and interpreted this as partly due to human disturbance preventing pollinators from visiting the flowers. Similarly, in the Huanglong National Park, the combination of the habitat edge created by the provision of the pathway, and tourist activity on the path, may reduce bumblebee activity and alter bumblebee behaviour.

Longer-term studies would be necessary to determine whether the effects recorded here will eventually alter the sizes and spatial structure of these orchid populations (Cunningham, 2000; Wilcock and Neiland, 2002). Although rapid growth in ecotourism and in the interest of the public in visiting sites of natural beauty, such as the study site, are recent phenomena, nature reserves are becoming increasingly popular as tourist destinations (Eagles, 2002). The results of this study suggest that, when nature reserves are designed to facilitate public access, great care must be taken to ensure that the facilities provided, and the presence of visitors, do not adversely affect the species of interest and conservation concern that are the reasons for their visits, or the vital interactions between these species and those on which they depend.

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