

Photosynthesis in Relation to Reproductive Success of *Cypripedium flavum*

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• **Background and Aims** *Cypripedium flavum* is a rare, endemic alpine slipper orchid of China, which is under threat from excessive collection and habitat changes. Conservation and re-introduction of *C. flavum* is restricted by lack of knowledge of the plant's photosynthesis and how that affects reproductive success. The hypothesis is tested that reproductive success is determined by photosynthetic production.

• **Methods** To understand the photosynthetic characteristics and adaptation of *C. flavum* to alpine environments, and the relation to reproductive success, measurements were made at four field sites with varying degrees of forest cover in the Hengduan Mountains, south-west China.

• **Key Results** Both photosynthetic capacity and reproductive traits of *C. flavum* are affected by light availability. Photosynthetic rate (A) is greatest around noon, following the pattern of photosynthetically active radiation (PAR) at all sites. *Cypripedium flavum* has highest daily mean photosynthetic rate (A_{daily}) and light-saturated photosynthetic rate (A_{max}) under a half to a third of full sunlight. High radiation decreased A . However, the optimum temperature for photosynthesis was similar (18–20 °C) at all sites.

• **Conclusions** The quotient of daily mean photosynthetic rate to light saturated photosynthesis ($A_{\text{daily}}/A_{\text{max}}$) is positively correlated with the ramet number m^{-2} and percentage of fruiting of *C. flavum*. The $A_{\text{daily}}/A_{\text{max}}$ ratio is a useful proxy for evaluating reproductive success of *C. flavum*.

Key words: *Cypripedium flavum*, photosynthesis, light availability, plant reproductive success.

INTRODUCTION

The genus *Cypripedium* comprises 49 species, which are widely distributed throughout Northern America, Eastern Asia and Europe. One of the most important distribution regions of the genus is the Hengduan Mountains of south-western China, where 14 species occur, mainly found at altitudes above 2700 m (Lang, 1990). Many *Cypripedium* species have ornamental and medical value. Because large-scale cultivation of these plants under artificial conditions is not economically feasible, wild populations of *C. flavum* are the major source of material for horticulture. In recent years, ecological disturbance, unscientific and uncontrolled collection, tourism and increasing grazing pressure has resulted in considerable decline of *Cypripedium* populations in the Hengduan Mountains (Cribb and Sandison, 1998; Kull, 1999). In order to conserve and cultivate *Cypripedium* species, knowledge of the optimal growth conditions is required. Such information may help to develop effective ways to restore the natural habitat of *Cypripedium* or to introduce these species into new environments. Although there are data regarding the general ecological preferences of several species (Primack and Hall, 1990; Primack and Stacy, 1998), there is still lack of detailed physiological information for most (Kull, 1999; Weng *et al.*, 2002). Better understanding of the physiology of *Cypripedium* species may aid their cultivation, which is still difficult.

Plants of *Cypripedium* species are long-lived (many survive more than 30 years) and generally flower in 6–10 years from seeds (Kull, 1999). In natural populations, the overall mean percentage of fruiting of *Cypripedium* species is very

low (Kull, 1998; Primack and Stacy, 1998). This may depend on the environment, as flower numbers and fruit set vary greatly in different habitats. In addition, large flowering populations may improve the reproductive success, as appears to be the case for *C. acaule* (Davis, 1986). Most *Cypripedium* species grow in the forest understory; therefore they may suffer from inadequate light due to competition with tall plants (Kull, 1999). Open vegetation, with greater light penetration, enables *Cypripedium* species to intercept more light, which is beneficial for growth and reproduction (Kull, 1998).

It is commonly assumed that plant physiological adaptations to particular environments confer a reproductive advantage (Arntz, 1999). Photosynthetic activity relates to plant productivity and species success (Nagel and Griffin, 2004). By examining the way in which the photosynthetic processes of a species are adapted to diverse environments, the links between growth, production, reproduction and climate can be assessed (Luoma, 1997), and the effect of demand by developing seeds and fruits on the photosynthetic activity of adjacent leaves analysed (Lehtilä and Syrjänen, 1995; Obeso, 2002). Instantaneous photosynthetic rate is not always a reliable predictor of plant growth as it is very variable, responding to seasonal and diurnal changes in environmental conditions. However, plant growth can be more accurately predicted when photosynthesis is considered together with patterns of dry matter allocation (Dijkstra and Lambers, 1989), although the direct and indirect effects of photosynthesis on growth and reproduction remain to be evaluated for particular species (Arntz *et al.*, 1998).

We hypothesize that higher photosynthetic rate is beneficial to reproductive success of *Cypripedium*. In order to

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evaluate the effect of environment on photosynthesis of *Cypripedium* species and to examine the relationships between photosynthesis and reproductive success, *C. flavum* plants growing in four different habitats were studied, including photosynthetic characteristics as well as reproductive traits. The long-term aim of this study is to provide information about the mechanisms and physiological parameters that may be important for successful reproduction of *C. flavum* and its cultivation and management.

MATERIALS AND METHODS

Species description and habitat

Cypripedium flavum grows in rocky and grassy places in sparse woods, alpine meadows or margins of forests at altitudes of 1800–3700 m in the west of China, including Yunnan, Sichuan, Gansu, Hubei and Xizang (Chen, 1999). In the Hengduan Mountains, annual mean temperature and precipitation are 5.4 °C and 624.8 mm (30-year mean), respectively. The climate is seasonal, with 87 % of annual rainfall occurring from May to October, while the dry season lasts from November to April. *Cypripedium flavum* grows on brown soil with abundant humic matter, and pH 6.1–6.8. It grows to 35–45 cm high, with 6–10 leaves produced from a rhizome. The growing period is about 140 d in a year. The seedling emerges above ground in mid-May and flowers appear in June. A single flower is borne on a scape arising between the basal leaves and subtended by a large, leaf-like bract. The plant sets fruit between July and September and it becomes dormant in early October. The capsule contains abundant dust-like seeds (about 6000–17 000 seeds).

Study sites

Plants were studied at four sites; the number of flowers and fruit set were significantly different in these habitats, where the largest environmental difference was light in the understorey due to differences in the density of the forest canopy. Sites were A (99°43.097'E, 28°11.399'N), B (99°33.463'E, 27°55.276'N), C (99°50.101'E, 27°47.760'N) and D (99°57.752'E, 27°36.568'N) at altitudes of 2910 m, 3260 m, 3450 m and 3360 m, respectively. Soil type, air temperature and water availability are very similar at the four sites.

Measurements of gas exchange in the field

Gas exchange of *C. flavum* was measured at the four sites in June 2003 (the flowering time). For each site, ten seedlings were selected and labelled for measurement.

Diurnal photosynthetic time-courses were measured at the sites on June 3–16 in 2003. Eight fully expanded leaves at each site were randomly selected from previously labelled seedlings; they were measured from 0800 h to 1800 h on a clear day. After steady-state of gas exchange had been achieved, photosynthetic rate (A), intercellular CO₂ concentration (C_i), leaf temperature (T_l) and photosynthetically active radiation (PAR) were measured using a portable infra-red gas exchange system with a leaf chamber

type PLC-B (CIRAS-1, PP Systems, UK) in an open-system configuration. In addition, a Li-188 quantum sensor, thermometer and hygrometer recorded, respectively, irradiance, air temperature and relative air humidity hourly. The diffuse radiation transmission coefficient and solar beam transmission coefficient were measured using a digital plant canopy imager (CI-110, CID, USA) at midday.

Light-saturated photosynthetic rates (A_{max}) were measured between 0900 h and 1200 h at ambient CO₂ partial pressure, air temperature 20 °C and PAR 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which was above the light-saturation point for *C. flavum*. Eight fully expanded leaves were measured at each site.

The photosynthetic response to light, CO₂ and temperature at sites A, B, C and D were measured on 11, 16, 17 and 20 June 2003, respectively. Photosynthetic responses of mature leaves to light were measured at 14 light intensities at each study site. The CO₂ concentration in the leaf chamber was 350 $\mu\text{mol mol}^{-1}$ and temperature 20 °C. Leaves were acclimated to PAR of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ before measurements. After the initial measurement at 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, light intensity was increased in 13 steps and photosynthetic rates were recorded. The CO₂ response curves of photosynthesis were determined with a range of CO₂ concentrations (0–2000 $\mu\text{mol mol}^{-1}$) at a light intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature of 20 °C. After the initial measurements at 2000 $\mu\text{mol mol}^{-1}$, CO₂ concentration was reduced in steps and photosynthesis recorded after 2–3 min acclimation at each concentration. Three leaves were measured at each study site. Curve-fitting software (Sigmaplot for Windows 8.0) was used to analyse both the $A-C_i$ and $A-PAR$ responses using a three-component exponential function (Watling *et al.*, 2000):

$$A = a(1 - e^{-bx}) + C \quad (1)$$

where A is photosynthetic rate, x is C_i or PAR , and a , b and C are constants. Using this equation, the carboxylation efficiency (CE) was estimated as the initial gradient of the $A-C_i$ curves (0–200 $\mu\text{mol mol}^{-1}$), and the apparent quantum yield (AQY) was calculated as the initial slope of the $A-PAR$ curves in the range 0–400 $\mu\text{mol mol}^{-1}$, following Swanborough *et al.* (1997).

The dependence of net photosynthetic rate on temperature was measured between 0900 h and 1100 h to avoid high temperatures at midday, and provided a temperature range of 10–35 °C. During measurements, the CO₂ concentration and light intensity were 350 $\mu\text{mol mol}^{-1}$ and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Three leaves were randomly selected and measured at each site. A second-degree polynomial equation was fitted to obtain the optimal temperature for photosynthesis.

Chlorophyll fluorescence of mature *C. flavum* leaves was determined using a pulse amplitude modulated fluorometer (FMS 2, Hanstech, UK). After adaptation for 25–30 min in the dark, the F_v/F_m ratio ($F_v/F_m = 1 - F_o/F_m$) was used to estimate photoinhibition of PSII. A second-degree polynomial equation was used to describe the relationships between F_v/F_m and temperature, and PAR .

In each population at each site, counts were made in six 5 × 5 m plots of the number of ramets and flowers during the

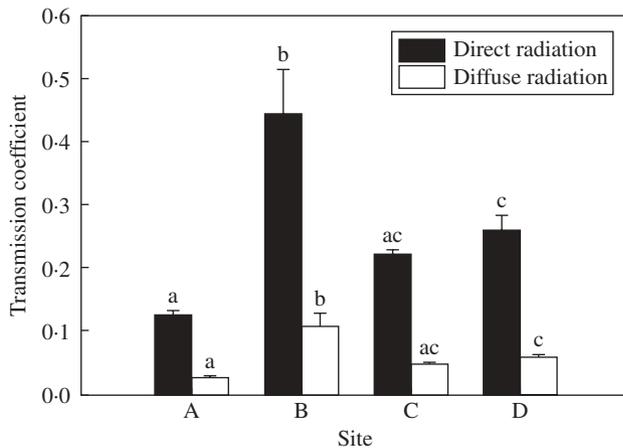


FIG. 1. The diffuse radiation transmission coefficient and solar beam transmission coefficient in the understorey at the four experimental sites. Error bars are the 95 % confidence interval of the mean, $n = 10$. Different letters indicate significant differences between means ($P < 0.05$).

flowering period (June 2003), and the number of ramets and fruits during the fruiting period (September 2003). Because each ramet only had a single flower, the percentages of flowering and fruiting were calculated as the ratio of number of flowering ramets and fruiting ramets to total ramets in each plot.

Statistical analyses

Statistical analysis was conducted using SPSS for Windows version 10.0 (SPSS, USA). Differences between physiological variables were determined using analysis of variance (ANOVA) and LSD tests for multiple comparisons. The relationships between photosynthetic parameters and reproductive traits were assessed using regression analysis.

RESULTS

Environmental conditions in the understorey

The transmission coefficients for diffuse radiation show the differences between the canopies at the four sites (Fig. 1) and are related to the quantity of light reaching the herb layer. Site B had the greatest direct and diffuse radiation ($P < 0.01$, $n = 10$), while site A had the lowest. Canopies create complex light dynamics (sun flecks) in the understorey due to the distribution and movement of foliage. Diurnal changes of PAR in the understorey at the four sites were not uniform. The daily PAR averaged 423 ± 71 , 785 ± 70 , 497 ± 53 and $482 \pm 59 \mu\text{mol m}^{-2} \text{s}^{-1}$ between 0800 h and 1800 h at site A, B, C and D, respectively. Minimum mean PAR was at site A, while maximal mean PAR was at site B. However, humidity and air temperature did not vary significantly between the four sites (Fig. 2).

Diurnal variation of photosynthesis

The average A of leaves at all sites increased rapidly with the increasing of PAR after 0800 h (Fig. 2), reaching maximum values around noon for sites B and C, and at 1400 h

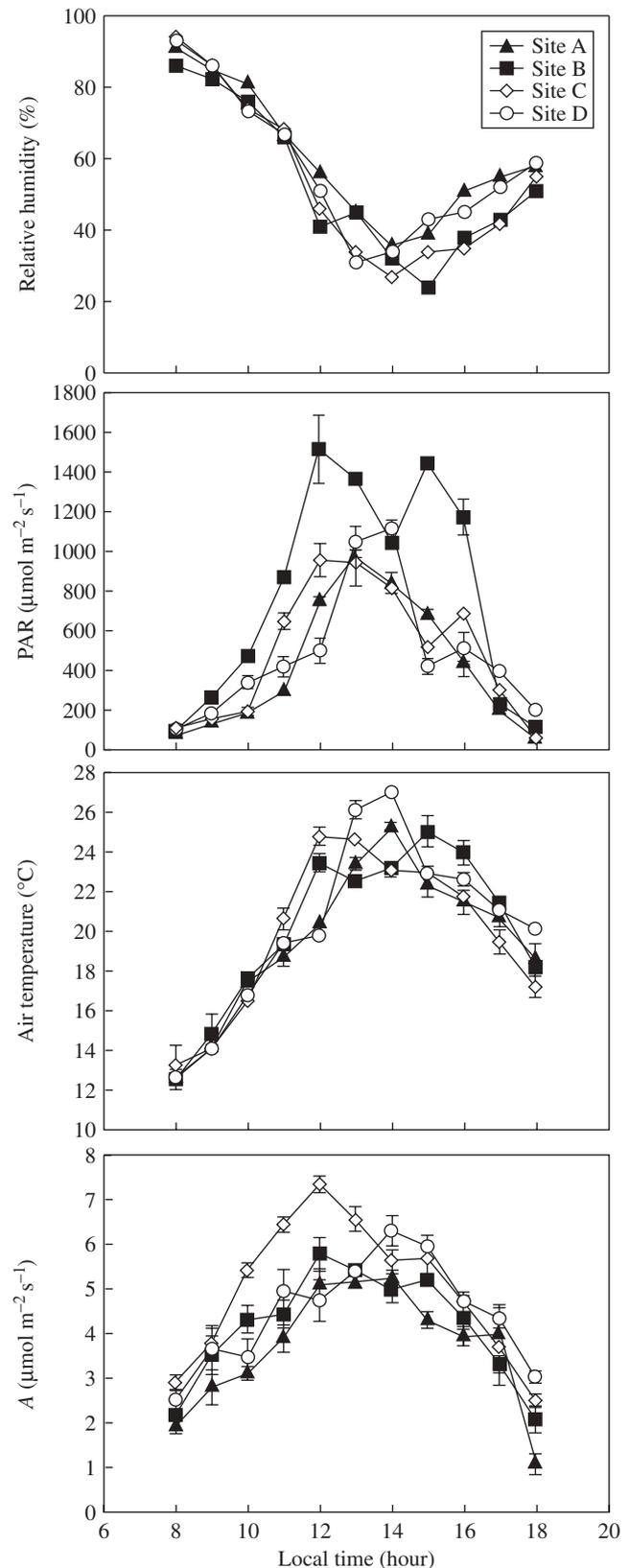


FIG. 2. Diurnal course of relative humidity, PAR, air temperature and photosynthesis for *C. flavum* at the study sites. Each point is the mean of five measurements. Error bars represent ± 1 s.e.

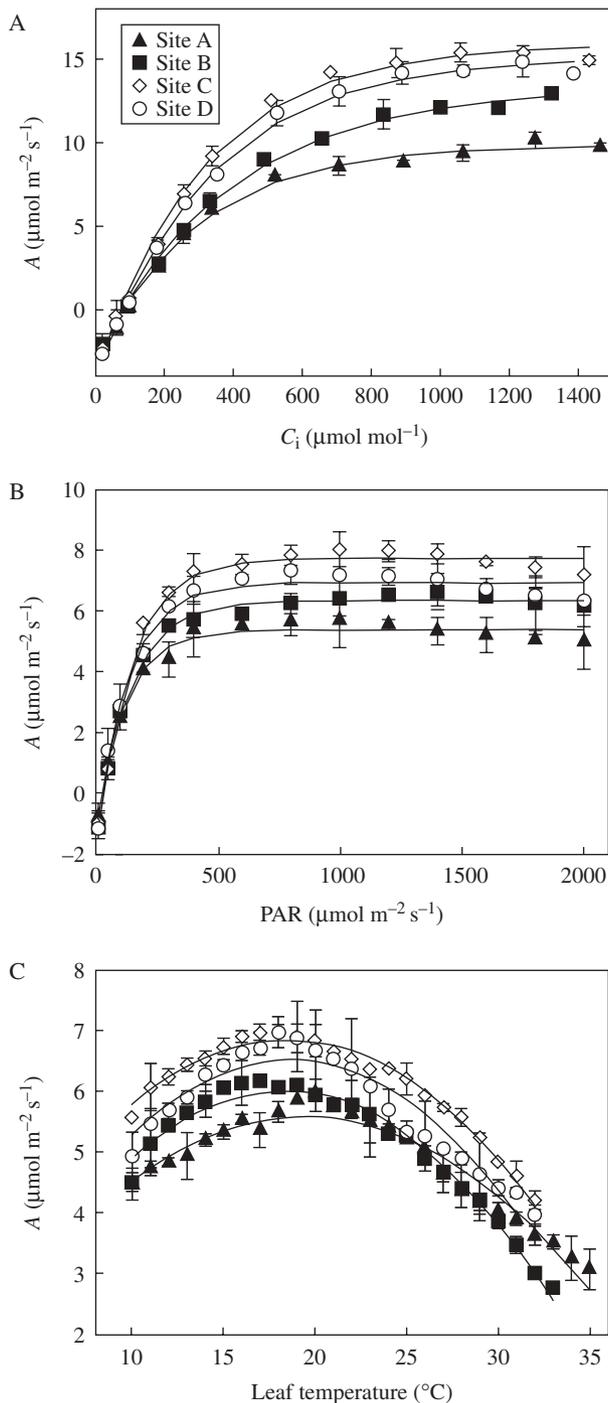


FIG. 3. Photosynthetic responses of *C. flavum* to (A) CO_2 ; (B) light; and (C) temperature at the study sites. Vertical bars s.e. of means ($n = 3$).

for sites A and D. There was no midday depression of photosynthesis at any site. The plants at site C had the highest A and A_{max} , while those at site A had the lowest ($P < 0.05$).

Effects of CO_2 , light and temperature on photosynthesis

The patterns of the A - C_i curves were similar for plants from all four sites (Fig. 3A). The photosynthetic CO_2

compensation points of the four populations varied from 72.9 to 83.5 $\mu\text{mol mol}^{-1}$ and were not significantly different ($P > 0.05$). According to the slopes of A - C_i curves in Fig. 3, carboxylation efficiencies (CE) of *C. flavum* in the four populations were significantly different ($P > 0.05$), being were 0.0278, 0.0237, 0.0362 and 0.0309 mol mol^{-1} for sites A-D, respectively.

The photosynthetic responses to light at the four sites are shown in Fig. 3B. The apparent quantum yield (AQY) of *C. flavum* was 0.0202, 0.0227, 0.0282 and 0.0257 $\text{mol CO}_2 \text{ mol photon}^{-1}$ at sites A-D, respectively. The plants at site C had the highest AQY, and it was lowest at site A ($P < 0.05$). There were no significant differences in photosynthetic light compensation points between populations (range 25.5–32.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$), but the photosynthetic light saturation points (LSP; PAR to achieve 99 % of A_{max}) were significantly different. *Cyripedium flavum* at site B had the highest LSP (700 $\mu\text{mol m}^{-2} \text{s}^{-1}$), while the lowest value (594 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was at site A. At values of PAR above 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the photosynthetic rate of *C. flavum* decreased gradually.

Photoinhibition of photosynthesis of *C. flavum*, indicated by smaller values of F_v/F_m , decreased linearly with increasing PAR ($R^2 = 0.58$, $P < 0.01$, $n = 120$) and F_v/F_m was curvilinearly related to temperature ($R^2 = 0.61$, $P < 0.01$, $n = 120$), as shown in Fig. 4. High temperature and high PAR inhibited the photosynthesis of *C. flavum*. When the leaf temperature was above 25 $^{\circ}\text{C}$, the photosynthetic rate decreased sharply. There were no significant differences in optimum temperature for photosynthesis (T_{opt}) between plants from the four sites (Fig. 3C).

Reproductive traits of different populations

The number of ramets, flowers and fruit per m^2 at the four sites are given in Fig. 5. The flowering percentage at sites A-D was 36.9 ± 8.7 , 47.0 ± 7.5 , 53.3 ± 6.7 and 42.6 ± 3.0 %, and the ratio of fruit-set was 2.9 ± 0.6 , 4.7 ± 1.1 , 9.2 ± 1.1 and 4.2 ± 0.8 %, respectively. The flowering percentage was not significantly different, but the population at site C had the highest percentage of fruiting among the sites ($P < 0.01$, $n = 6$).

The relationships between photosynthetic parameters and reproductive traits were described by second-order polynomial equations (Fig. 6). There is linearly relationship between daily mean A and the number of ramet per m^2 ($R^2 = 0.68$, $P < 0.01$, $n = 24$) and percentage of fruiting ($R^2 = 0.56$, $P < 0.01$, $n = 24$). The ratio of daily mean photosynthetic rate to light-saturated photosynthesis ($A_{\text{daily}}/A_{\text{max}}$) was 64.0, 67.9, 72.1 and 65.9 % at sites A-D, respectively. The ratio was closely correlated with the ramet number per m^2 ($R^2 = 0.81$, $P < 0.01$, $n = 24$) and percentage of fruiting ($R^2 = 0.60$, $P < 0.01$, $n = 24$; Fig. 6).

DISCUSSION

Light availability under different forest understoreys

Light intensity at the ground surface is affected by the forest canopy cover, which creates complex light dynamics for

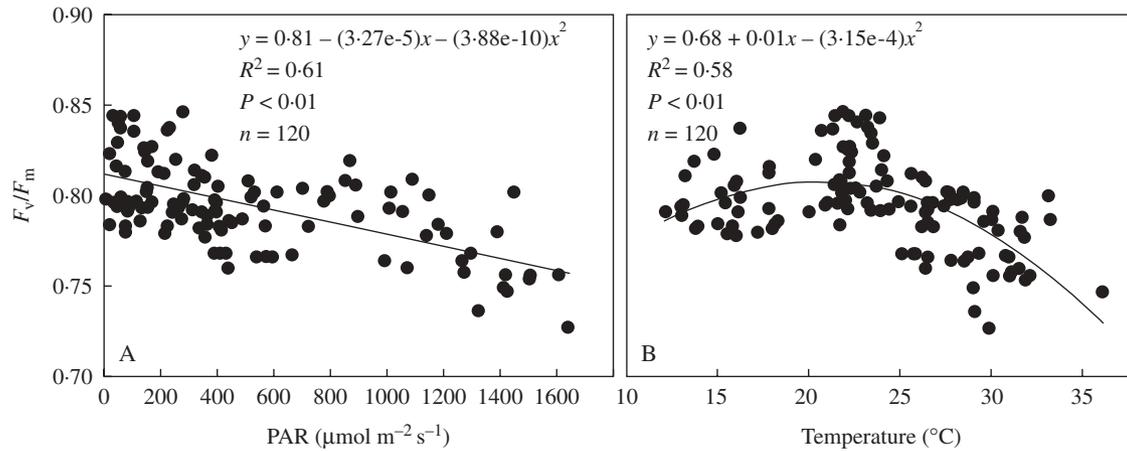


FIG. 4. The effects of (A) light and (B) temperature on the F_v/F_m ratio of *C. flavum* in the Hengduan mountains.

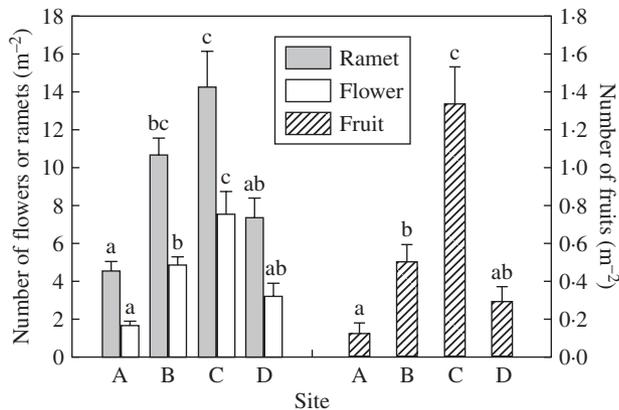


FIG. 5. The number of ramets, flowers and fruits per m^2 for *C. flavum* grown at the different study sites. Values are means of six replicates and vertical bars represent the s.e. Different letters indicate significant differences between sites at $P < 0.05$.

C. flavum due to the distribution and movement of foliage. Effective acquisition and use of light are critical, especially for plants inhabiting in the forest understorey, as shading may not be favourable to growth and reproduction (Hughes *et al.*, 1988). Seedlings of *C. flavum* under the sparsest forest (site B) received most direct and diffuse irradiation, followed by D, C and A.

Leaf photosynthetic traits in understorey environments

Light conditions affect the morphological and physiological characteristics of understorey plants. Plants of *C. flavum* at site C had the highest values for A_{max} , apparent quantum yield and carboxylation efficiency. Those at site A had the least. The photosynthetic light compensation points of *C. flavum* at the four sites were very low (ranging from $25.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at site A to $32.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ at site B); this is an important feature of the carbon balance as low LCP improves carbon gain in shade environments (Muraoza *et al.*, 2003).

Light is a limiting factor for the establishment of seedlings in populations of *C. calceolus* in Europe (Kull, 1998). Sites suitable for seedling establishment have more

extensive moss cover, more moisture, but less vascular plant cover and more intense light (Kull, 1998). The proportion of reproductive ramets is larger in more intense light, and in shady localities small clones dominate (Kull, 1999). A fraction of 1/14–1/20 of full light is optimal for *C. calceolus* in the taiga zone of Europe (Kull, 1995). The poor growth for *C. calceolus* in shade conditions may be due to this limited resource. In our study, the photosynthetic light-saturated points (LSP) of *C. flavum* were from 593 to $700 \mu\text{mol m}^{-2} \text{s}^{-1}$, which are equal to one-quarter to one-third of full sunlight. The seedlings at site B had the highest LSP due to the higher light intensity. *Cypripedium flavum* at site C had the highest photosynthetic potential due to suitable light. Because *C. flavum* is adapted to lower light conditions in the understorey, exposure to strong light (above $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) resulted in photoinhibition of photosynthesis. The value of A for the populations is depressed by the excess sunlight at site B and by shade condition at site A.

Relationship between photosynthetic capacity and reproduction

The growing season for *C. flavum* is short (about 140 d) in the Hengduan Mountains. In order to reproduce, *C. flavum* must complete the process from emergence, through flowering to fruiting in a short period, allowing sufficient time to store adequate energy for the next year. Shading may reduce the photosynthetic capacity and the ability of perennial species to store resources for subsequent growth and reproduction (Vallius, 2001). So the light availability under different forest canopy coverage would explain the differences in reproductive success of *C. flavum* at the different sites.

It is commonly assumed that instantaneous photosynthetic rates of leaves are the consequence of environmental factors, but they may also be affected by plant demand for assimilates from growth and seed production (Burton and Bazzaz, 1995; Tissue *et al.*, 1995; McAllister *et al.*, 1998). When growth is limited by light, water or nutrient availability, the morphological and physiological traits involved in the acquisition and allocation of limiting

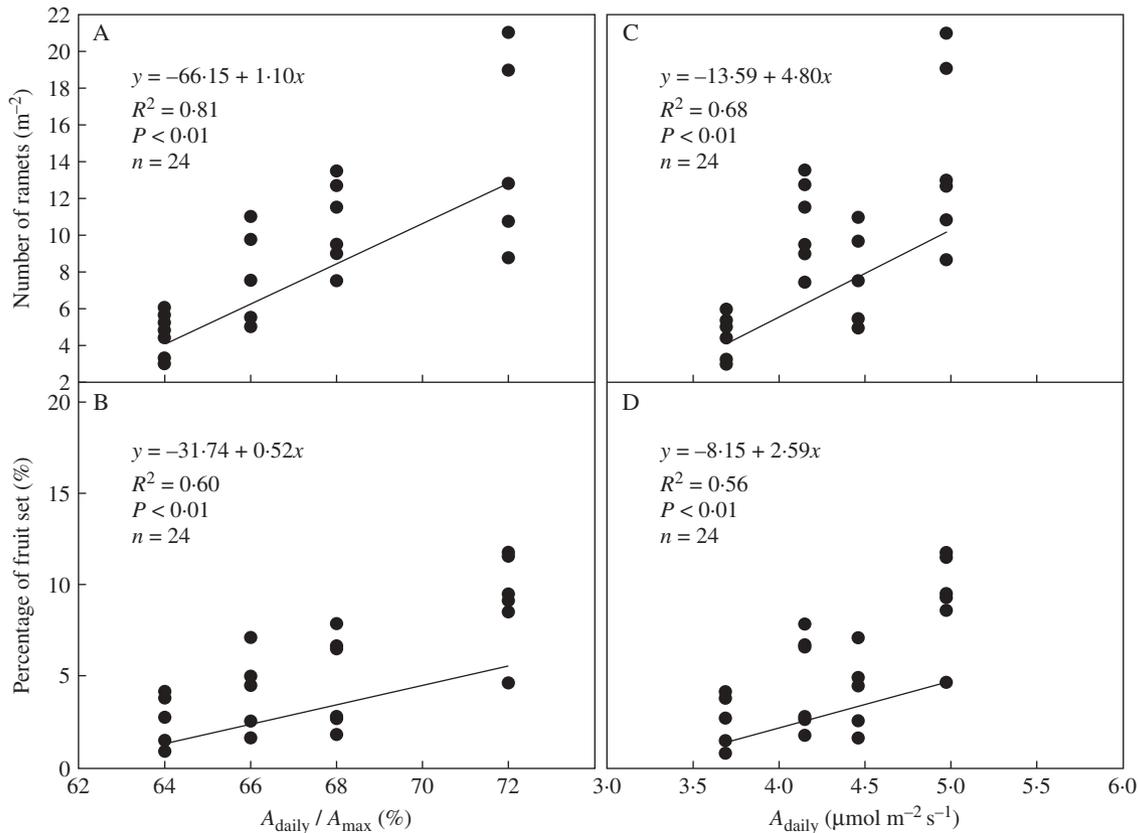


FIG. 6. The relationships between $A_{\text{daily}}/A_{\text{max}}$ and (A) the number of ramets per m^2 and (B) percentage of fruiting; and the relationships between daily mean A and (C) the number of ramet/ m^2 and (D) percentage of fruiting.

resources are expected to influence growth and production (Arntz, 1999), and thus affect populations and their reproduction. A higher photosynthetic rate should increase carbon gain and in turn increase accumulation of biomass, leading to an increase in fitness (Arntz *et al.*, 2000). Within a season, fruit production may be limited by pollinators, but the availability of resources ultimately limits fruit production and reproductive success (Snow and Whigham, 1989; Zimmerman and Aide, 1989; Sugiura *et al.*, 2001). In our study, the ratio of daily mean photosynthetic rate to light-saturated photosynthesis ($A_{\text{daily}}/A_{\text{max}}$) for *C. flavum* at sites A–D was 64.0, 67.9, 72.1 and 65.9%, respectively. The daily mean photosynthetic rate (A_{daily}) and $A_{\text{daily}}/A_{\text{max}}$ are closely correlated with the number of ramets per m^2 and percentage of fruiting (Fig. 6).

It is concluded that photosynthesis and thus reproduction of *C. flavum* are affected by light availability in the understory, supporting the hypothesis that higher photosynthetic rate increases reproductive success and fitness of *C. flavum*. The ratio $A_{\text{daily}}/A_{\text{max}}$ expresses the effect of the currently prevailing environment on photosynthesis, and because of the relationship with fruiting it can also be regarded as a proxy of reproductive success.

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