

Is Floral Longevity Influenced by Reproductive Costs and Pollination Success in *Cohniella ascendens* (Orchidaceae)?

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Received: 5 June 2007 Returned for revision: 4 July 2007 Accepted: 26 July 2007

- **Background and aims** Although studies have shown that pollen addition and/or removal decreases floral longevity, less attention has been paid to the relationship between reproductive costs and floral longevity. In addition, the influence of reproductive costs on floral longevity responses to pollen addition and/or removal has not yet been evaluated. Here, the orchid *Cohniella ascendens* is used to answer the following questions. (a) Does experimental removal of flower buds in *C. ascendens* increase flower longevity? (b) Does pollen addition and/or removal decrease floral longevity, and does this response depend on plant reproductive resource status?
- **Methods** To study the effect of reproductive costs on floral longevity 21 plants were selected from which we removed 50% of the developing flower buds on a marked inflorescence. Another 21 plants were not manipulated (controls). One month later, one of four flowers on each marked inflorescence received one of the following pollen manipulation treatments: control, pollinia removal, pollination without pollinia removal or pollination with pollinia removal. The response variable measured was the number of days each flower remained open (i.e. longevity).
- **Key Results** The results showed significant flower bud removal and pollen manipulation effects on floral longevity; the interaction between these two factors was not significant. Flowers on inflorescences with previously removed flower buds remained open significantly longer than flowers on control inflorescences. On the other hand, pollinated flowers closed much faster than control and removed-pollinia flowers, the latter not closing significantly faster than control flowers, although this result was marginal.
- **Conclusions** The results emphasize the strong relationship between floral longevity and pollination in orchids, as well as the influence of reproductive costs on the former.

Key words: *Cohniella ascendens*, floral longevity, flower bud removal, pollination, pollinia removal, reproductive costs.

INTRODUCTION

Floral longevity plays a central role in plant reproductive ecology as it affects the number of pollinator visits, the amount and quality of pollen received and disseminated, as well as floral display size (Primack, 1985; Harder and Johnson, 2005), all of which determine plant reproductive assurance and overall fitness (Rathcke, 2003). The amount of time a flower remains open depends on biotic and abiotic environmental factors present at a given site (e.g. water availability, pollinator abundance; Ashman and Schoen, 1994, 1996), as well as on species-intrinsic factors which determine the way in which plants respond to the former (e.g. growth habit, life history; Primack, 1985). Both these factors combined, environmental and species-intrinsic, determine floral longevity through trade-offs such as that proposed between flower maintenance and construction (Ashman and Schoen, 1994; Schoen and Ashman, 1995).

Orchids usually exhibit long-lived flowers (1 week to months), and, to some extent, this is thought to be an evolutionary outcome of their specialized pollination systems and low pollinator visit rates (Primack, 1985; Stpczynska, 2003), which commonly results in them being pollen-limited (Tremblay *et al.*, 2005). Specifically, in the case of orchid species that grow in tropical

environments, pollen limitation is generally greater in these compared with orchids in temperate areas (Neiland and Wilcock, 1998; Tremblay *et al.*, 2005), and fluctuations in water availability and elevated temperatures will most likely translate into high reproductive costs. Based on this, orchid species found in these habitats might show particularly strong floral longevity responses to reproductive costs and pollination success, and the interaction between these two effects might indicate potential changes in floral longevity responses to pollen addition and/or removal due to differences in plant reproductive resource status.

Cohniella ascendens is a common orchid found in seasonally dry tropical forests of the Yucatán Peninsula and, like most orchids, it is pollen limited (Parra-Tabla *et al.*, 2000). It flowers during the dry season (January to April), when precipitation is at its lowest and monthly temperature averages start to rise (25–27 °C; García 1988). These abiotic conditions, together with its low reproductive success, make of *C. ascendens* an ideal system to test hypotheses on pollen manipulation (environmental factor) and reproductive cost (species-intrinsic factor) effects on floral longevity, as well as their interaction. Furthermore, although many studies have shown that pollination decreases floral longevity in orchids (e.g. Proctor and Harder, 1995; Clayton and Aizen, 1996; van Doorn, 1997; Martini *et al.*, 2003; Stpczynska, 2003), much less

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attention has been paid to the relationship between reproductive costs (e.g. flower construction, maintenance) and floral longevity (exceptions are Holtsford, 1985; Ashman and Schoen, 1997). For these reasons, *C. ascendens* was chosen to answer the following questions. (a) Does experimental removal of flower buds in *C. ascendens* (i.e. reduced flower construction-maintenance costs) increase flower longevity? (b) Does pollen addition and/or removal decrease floral longevity, and is this effect influenced by plant reproductive resource status? By addressing such questions, this study contributes to the understanding of phenotypic plasticity in floral longevity under changing pollinator and plant resource status conditions.

METHODS

Study species and site

Cohniella ascendens Lindey (Orchidaceae; before *Oncidium ascendens*, Carnevali *et al.*, 2001) is widely distributed in Mexico, and is found both in evergreen as well as subdeciduous forests of the Pacific and Atlantic coasts, including the Yucatán Península (Andrews and Gutierrez, 1988; Olmsted and Gómez-Juárez, 1996). In the state of Yucatán, it is a common orchid in seasonally dry tropical forests, and is usually found growing as an epiphyte on trunks of *Acacia gauderi*, *Enterolobium cicloparum* and *Gymnopodium floribundum* (Parra-Tabla *et al.*, 2000), with each plant producing one or two pseudobulbs a year. *Cohniella ascendens* is self-incompatible, which is a common condition for other species of this genus (Gill, 1989; Endress, 1994), although spatial variation in this condition has been observed for the study species (V. Parra-Tabla, unpubl. data). An adult plant produces one or two, 50-cm-long inflorescences during the reproductive season, each one typically with 6–18 small (1.5 cm in diameter) yellowish brown-dotted nectarless flowers. Each flower contains two pollinia, and remains open from 6 to 10 d; flowering occurs during the dry season (January to April). Its primary pollinators in Yucatán are native bee species of the genus *Trigona* (Parra-Tabla *et al.*, 2000) which collect resins from the flower to build their nests (Wille, 1983; Gill, 1989; Dressler, 1993). In addition, other floral visitors from the genus *Centris* have been observed (V. Parra-Tabla, unpubl. data).

The study site was located within the limits of the Campus de Ciencias Biológicas y Agropecuarias of the Universidad Autónoma de Yucatán (Yucatán, México; 20°52'4"N, 89°37'24"W). Study plants were found growing in a matrix of seasonally dry tropical forest approx. 20 years old. The site is 9 m a.s.l. and climate is tropical warm humid, subhumid (Aw, modification of Köpen by García, 1988). Average temperature is 26 °C and mean annual precipitation is 935 mm (García, 1988).

Field work and treatments

In early February 2002, at the onset of the reproductive season of *C. ascendens*, 42 adult plants were selected and one inflorescence was chosen and flagged on each plant.

All marked inflorescences were matched for approximately the same number of flower buds. To study the effects of flower construction (and maintenance) costs on floral longevity, a flower-bud removal experiment was conducted that involved randomly selecting 21 of the previously mentioned plants from which 50 % of the flower buds were removed on a marked inflorescence (randomly chosen along the stalk), during the earliest possible bud developmental stage in order to minimize damage. The remaining 21 plants were controls, and were not subject to flower bud removal. Flower bud removal has been used before to evaluate resource trade-offs between flower construction costs and other reproductive structures (e.g. fruits) (see Silvertown, 1987; Herrera, 1991; Ashman and Hitchens, 2000). Plants that had their flower buds removed did not differ significantly from unmanipulated plants in number of leaves (50 % flower bud removal = 4.35 ± 0.32 , 0 % = 4.32 ± 0.23 ; $t_{40} = 0.35$, $P = 0.72$). In addition, photosynthetic photon flux density (400–700 nm) was measured next to each plant with a line quantum sensor (LI-191SA; LICOR, Lincoln, NE, USA), from 1100 to 1500 h on three consecutive days and no significant differences were found between treatment groups (50 % = $74.4 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 46.60$; 0 % = 64.7 ± 32.2 ; $t_{40} = 0.06$, $P = 0.94$). Finally, inflorescences were followed throughout the field season to verify that flower bud removal did not result in parasitism of affected tissue (which it did not).

One month after removing flower buds, using the same 42 plants, one of four intact, newly opened flowers on each marked inflorescence was randomly selected to receive one of the following pollen manipulation treatments: control or no manipulation (C), pollinia removal (R), pollination without pollinia removal (POL) or pollination with pollinia removal (POL + R); the latter two involved cross pollinations. Treatments were applied between 0500 and 0600 h (i.e. when flowers open). Inflorescences were not bagged to exclude pollinators, although daily censuses from 0600 to 1200 h showed that sampled flowers were not visited throughout the experiment and, most importantly, that flowers on study plants did not show any visual signs of pollination other than that artificially-induced; this is not unusual as visit rates have been shown to be extremely low for the study species (fruit set < 4 %; Parra-Tabla *et al.*, 2000). Plants were visited daily subsequent to treatment initiation until all sampled flowers had closed. The response variable measured was the number of days each flower remained open (i.e. longevity).

Statistics

Data were analysed with a log-linear model in SAS (PROC GENMOD; SAS Institute, 2002) for which flower bud removal and pollen manipulation were used as independent factors, as well as their interaction, influencing the number of days a flower remained open. The interaction term might provide potential insights into changes in floral longevity responses to each pollen manipulation treatment due to plant reproductive resource status differences. Non-significant factors were removed from the model in a backward fashion. Type III analysis was used, and

pre-planned contrasts were conducted in a pairwise manner between treatment levels of significant main effects using the ESTIMATE option. Flower vertical position on the inflorescence stalk (tip, centre or base), which was assessed by mentally dividing the inflorescence into three segments of equal length, was used as covariate. Preliminary results showed that the covariate was not significant (neither were its interactions with the main effects) and, for this reason, it was not included in the final model reported here.

RESULTS

The log-linear model showed significant flower bud removal ($F_{1,174} = 8.55$, $P = 0.003$, Fig. 1A) and pollen manipulation effects ($F_{3,174} = 665.75$, $P < 0.0001$; Fig. 1B) on the number of days a flower remained open, after removal of the non-significant flower bud removal \times pollination treatment interaction ($F_{3,171} = 1.39$, $P = 0.70$). See Table 1 for descriptive statistics. Pre-planned contrasts showed that flowers on inflorescences with previously removed flower buds remained open significantly longer than flowers on control inflorescences (50 % bud removal mean \pm s.e.; 5.09 d \pm 0.89; 0 %, 4.20 \pm 0.89; $\chi^2 = 8.52$, $P = 0.003$; Fig. 1A). On the other hand, pre-planned contrasts between pairs of pollen manipulation treatment levels showed the following differences (Fig. 1B): average flower longevity values for flowers subject to POL and POL + R treatment levels did not differ significantly (mean = 1 d \pm 0.001 s.d. in both cases; $\chi^2 = 0.01$, $P = 0.98$) and showed the lowest longevity values by far, differing significantly from control ($\chi^2 \geq 214$, $P < 0.0001$ in both cases), and removed-pollinia flowers ($\chi^2 \geq 194$, $P < 0.0001$ in both cases). Removed pollinia flowers (R = 8.41 \pm 3.22), did not show significantly lower longevity compared with control flowers, although this result can be considered marginal ($\chi^2 = 2.79$, $P = 0.09$). Control flowers showed the greatest average longevity value (C = 9.52 \pm 2.47).

DISCUSSION

Results from this study show that there was an overall strong effect of pollinia deposition on floral longevity in

C. ascendens, a typical response found in orchids (e.g. Ackerman, 1989; Proctor and Harder, 1995; Clayton and Aizen, 1996; Martini *et al.*, 2003; Stpiczynska, 2003). Pollinated flowers, regardless of having their pollinia removed or not, closed much faster than control or removed-pollinia flowers. On the other hand, pollinia removal only caused a marginal decrease in floral lifespan compared with control flowers. Similar results have been found in other orchid species such as *Encyclia krugii* (Ackerman, 1989), *Calypso bulbosa* (Proctor and Harder, 1995), *Chloraea alpina* (Clayton and Aizen, 1996) and *Mistacidium venosum* (Luyt and Johnson, 2001).

Differences in floral lifespan responses to pollinia removal *vs.* deposition may be explained based on the fitness benefit of remaining open in each case. Clayton and Aizen (1996) suggest that a flower that has had its pollinia removed remains open more time than a pollinated one because it still has a large portion of fitness benefit that remains to be gained (i.e. can still be pollinated). On the other hand, a flower that has been pollinated will close faster because the benefit of closing (e.g. securing fruit formation) outweighs that of remaining open. This theoretical framework fits orchid reproduction especially well given that they are pollen-limited and depend on long-lived flowers to compensate for low visitation rates (Ackerman, 1986). In addition, Clayton and Aizen (1996) point out that the physiological signals provided by pollen removal are weaker and less numerous than those associated to pollen deposition, many of which have a strong effect on flower attributes such as longevity (see also Arditti and Flick, 1976). On the other hand, based on the theory of sexual selection in plants (Wilson, 1979), in order to maximize male reproductive success (which is typically pollinator-limited), selection would favour flowers which have had their pollinia removed to remain open in order to maximize the male function, as well as to increase pollinator visitation rates at the plant level (e.g. via greater flower display size) (Parra-Tabla and Vargas, 2007).

Proctor and Harder (1995) suggest that flowers that have had their pollinia removed should take more time to close in deceptive orchids than in rewarding ones because the female function is less easily satisfied than the male function in the former. Furthermore, flowers with rewards are

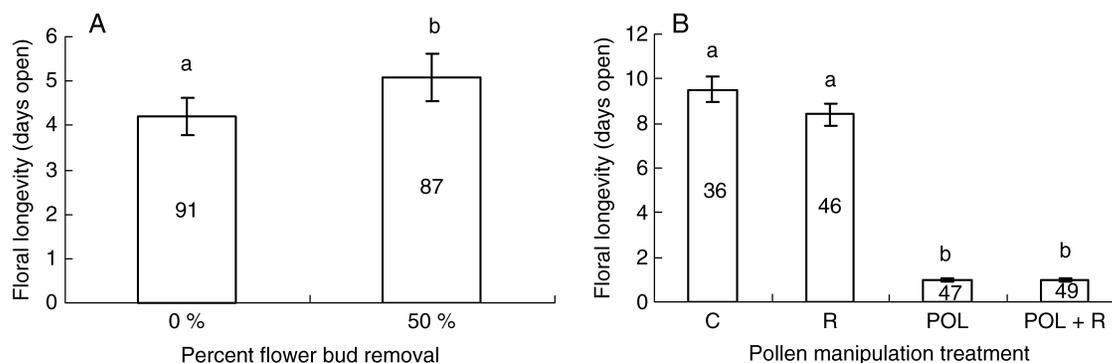


FIG. 1. Flower bud removal (A) and pollen manipulation treatment effects (B) on floral longevity in *C. ascendens*. Values represent means \pm s.e. Different letters indicate significant differences ($P < 0.05$), and numbers inside bars are sample sizes. C, No manipulation; R, removed pollinia; POL, pollination with no pollinia removed; POL + R, pollination with pollinia removed.

TABLE 1. Mean number of days *C. ascendens* flowers remained open under each pollen manipulation treatment for each inflorescence flower bud removal level.

Pollen treatment	Flower bud removal			
	0 %		50 %	
	<i>n</i>	Mean \pm s.e.	<i>n</i>	Mean \pm s.e.
C	19	8.68 \pm 0.74	17	10.47 \pm 0.89
R	23	7.34 \pm 0.65	23	9.47 \pm 0.65
POL	24	1.00 \pm 0.001	24	1.00 \pm 0.001
POL + R	25	1.00 \pm 0.001	23	1.00 \pm 0.001

n, Sample size for each treatment combination; C, no manipulation; R, removed pollinia; POL, pollination, no pollinia removal; POL + R, pollination, with pollinia removal.

probably physiologically more expensive than deceptive ones and would close sooner in order to reduce flower maintenance costs, such as nectar production (Southwick, 1984). Evidence from previous studies focusing on pollen removal effects on floral longevity in deceptive orchid species have usually failed to find a significant effect (e.g. Ackerman, 1989; Proctor and Harder, 1995). On the other hand, two other studies have tested for such effects in rewarding orchids; in one of them, Luyt and Johnson (2001) found that pollinia removal significantly decreased floral longevity in *Mystacidium venosum*. These results show some degree of contrast in floral longevity responses to pollinia removal between rewarding and deceptive orchid species, fitting predictions by Proctor and Harder (1995). Nonetheless, the pollinia removal effect on floral longevity observed for *C. ascendens* in this study was not as clear as that found in *M. venosum* (C vs. R flowers: $P = 0.09$), and in a study by Martini *et al.* (2003) no effect of pollinia removal on floral longevity was found in the rewarding orchid *Gongora quinquenervis*, which is inconsistent with the predictions of Proctor and Harder. Furthermore, two other studies have found pollinia removal to significantly decrease flower lifespan in the deceptive orchids *C. alpina* (Clayton and Aizen, 1996) and *Myrmecophila christinae* (V. Parra-Tabla *et al.*, unpubl. res.). Based on this, we suggest that in order to test the argument by Proctor and Harder, comparative studies on floral longevity responses across species with each pollination system (rewarding vs. non-rewarding) are needed, which in addition control for other factors such as habitat differences and phylogeny.

The other main point of discussion in this study has to do with reproductive costs and floral longevity. *Cohniella ascendens* flowers are small (approx. 1.5 cm in diameter) which suggests that they are cheap to build and maintain (Primack, 1985; Galen, 2000), and, in addition, no effect of flower position on flower longevity was found, pointing at reduced flower maintenance costs (if such costs were high, distal flowers should be more resource-limited; Stpiczynska, 2003). However, we *did* find that flower bud removal (i.e. greater resource availability) significantly increased floral longevity, and this is one of the few studies to offer evidence of reproductive costs affecting

floral longevity (see below). Although the physiological cost of resin production is ignored, it is possible that the terpenes and oils that constitute such reward (Porto *et al.*, 2000) impose a significant flower maintenance cost for the plant. In addition, epiphytes typically undergo high rates of transpirational water loss making water conservation take priority over carbon gain (Zotz and Heitz, 2001; Zotz and Mikona, 2003), and this condition, although speculative, might translate into high reproductive costs in *C. ascendens*.

Although Wilcox-Wright and Meagher (2003) suggest that resource reallocation in response to seed predation influences floral longevity in *Silene latifolia*, as far as is known, the only two studies that have explicitly tested for a trade-off between flower longevity and reproductive output are those by Holtsford (1985) and Ashman and Schoen (1997) who manipulated floral longevity by controlling the amount of time before pollination in *Calochortus leichtlini* and *Clarkia tembloriensis*, respectively. Both studies found a trade-off between floral longevity and fruit and seed production given that the latter two decreased under a delayed pollination treatment. Likewise, results from the present study show a trade-off between floral longevity and flower construction–maintenance, although instead of manipulating longevity, which was the response variable in this case, we manipulated both reproductive costs and pollination success, in what we consider a novel experimental framework in floral longevity studies. Although a significant interaction between pollen treatment and resource status was not found, this term provides information on potential differences in floral longevity responses to pollen addition and/or removal across plant resource status conditions, thus bringing together pollination ecology and resource allocation theory in order to achieve a better understanding of floral lifespan variation, and its implications on plant reproductive success.

ACKNOWLEDGMENTS

We thank M. Sedgley and two anonymous reviewers who contributed with useful comments that improved the quality of this manuscript, P. Alonso for field work and data input, and C. Selem-Salas for literature search. This study was financed by CONACyT (project no. 32456).

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