

A Mechanism Facilitates Pollination due to Stigma Behavior in *Campsis radicans* (Bignoniaceae)

YANG Shu-Xiang, YANG Chun-Feng, ZHANG Tao, WANG Qing-Feng*

(College of Life Sciences, Wuhan University, Wuhan 430072, China)

Abstract: The precise factors affecting stigma behavior in *Campsis radicans* (L.) Seem. ex Bureau. remain unclear up to now. In this study mechanical touch, self- and cross-pollination, and pollination with variable amounts of pollen grains separately contacting with stigmas have been conducted to determine the exact factor affecting the stigma behavior. Results show that mechanical touch alone cannot make the stigmas close permanently. It is the adequate pollen (> 350) deposition that causes the stigma permanent closure, which is in accordance with previous reports that sufficient pollen grains are necessary for fruit development. In addition, the stigma behavior does not display differences when pollinated with cross- or self-pollen separately; both self and cross pollen grains can germinate and grow successfully. Our results cannot demonstrate that the stigma behavior in *C. radicans* is an outcrossing mechanism, but strongly indicate it acts as a mechanism to facilitate pollination, and then enhance the reproductive success.

Key words: stigma behavior; *Campsis radicans*; bilobed stigma; pollination

As an important reproductive structure, stigma plays an important role in reproductive success of flowering plants (Guo and Huang, 1999; Yang *et al.*, 2002). Moreover, several recent studies show that the movement of stigma can facilitate outcrossing and/or pollination (Fægri and van der Pijl, 1979; Fetscher and Kohn, 1999; Li *et al.*, 2001; Li *et al.*, 2004). Li *et al.* (2001) found that the stigma movement due to the flexible style in *Alpinia* obviously encouraged outcrossing. Fægri and van der Pijl (1979) demonstrated that the active stigma in *Iris* could enhance pollination efficiency by combing pollen off the pollinators. Li *et al.* (2004) also found that the stigma with the "comb like" fringe hairs in *Caesalpinia crista* could scrap off the pollen grains adhered on the abdomen of the insects into the stigmatic chamber. In another study, the behavior of bilobed stigma in *Mimulus aurantiacus* was considered as a mechanism to prevent the intrafloral selfing because the stigma would quickly close in response to touch by a pollinator (Fetscher and Kohn, 1999). The stigma in *Campsis radicans* (Bignoniaceae) also owns two active lobelets. Several hypotheses have been addressed to the significance of stigma closure: aiding pollen capture and receipt (Thieret, 1976); preventing pollen grains loss from stigmas (Bertin, 1982a); preventing intrafloral selfing (Newcombe, 1922; 1924; Lloyd and Yates, 1982; Ritland and Ritland, 1989); reducing interference between pollen receipt and export functions within the flower (Webb and Lloyd, 1986); and increasing pollen export (Fetscher *et al.*, 2002). A series of studies on

reproductive biology of *C. radicans* have been reported (Bertin, 1982a; 1982b; 1985; 1986; 1990a; 1990b; Bertin and Sullivan, 1988; Bertin *et al.*, 1989; Bertin and Peters, 1992; Prakash, 1994; Galetto, 1995), and the stigma behavior of *C. radicans* has been noticed and evaluated (Bertin, 1982a). However, the exact factors affecting stigma movement and the precise role of the stigma behavior in reproductive process have not been clearly evaluated. We hypothesize that two potential roles should be responsible for the significance of the stigma behavior: enhancing outcrossing and/or facilitating pollination. In this study, we try to ascertain the following objectives: (1) what causes the stigma open and closure? (2) do different pollen sources (self or outcross) show different effects on stigma behavior? (3) whether different pollen number show different effects on stigma behavior?

1 Materials and Methods

Campsis radicans (L.) Seem. ex Bureau. is a protandrous hermaphrodite, sprawling or climbing woody plant, with long, red, tubular corollas, nectary and no scent; usually cross-pollinated by hummingbirds and bees (Bertin, 1982a; 1988; 1989). The stigma has two lobelets, which is exerted beyond the anthers in most plants (Fig. 1). Five introduced *C. radicans* populations were selected in this study. All fieldwork was conducted in Wuhan, Hubei Province between May and October in 2002 and also in 2003. At least 30 inflorescences were selected randomly from different

individual plants in each population for artificial treatments.

All chosen flowers were previously bagged. Four kinds of artificial treatments were designed to the open virgin stigmas: (1) mechanical touch ($n=150$); (2) self-pollination ($n=150$); (3) cross-pollination ($n=150$); (4) pollination with variable amounts of pollen grains ($n=450$). In each treatment, we recorded the data as follows: whether the stigma closure or reopen and the time of the stigma closure or reopen.

All pollinated stigmas were collected and fixed in FAA. The amounts of pollen grains deposited on the stigmas in the fourth treatment were counted under the fluorescence microscope, a drop of 0.1% aniline blue in 0.15 mol/L K_2HPO_4 (pH 9.0) was added to each stigma on a microscope slide before counted. The flowers self- or cross-pollinated were collected separately after pollinated 0.5 h, 1 h, 2 h, 3 h, 24 h. Their pollen tube growths were observed under the fluorescence microscope.

2 Results

During the above study, we found that the anthers have dehisced before the flowers opened, while the bilobed stigmas mostly began to spread apart about two hours later. In general, the buds opened during 7:30 - 9:30 (a.m.), and the stigmas completely opened during 10:30 - 11:30 (a.m.). The open stigmas always kept open if not touched, even in the evening, till faded 3 to 4 d later.

The stigmas given the mechanical touch mostly would close between 40 s and 150 s, all of them would reopen



Fig.1. Flower of *Campsis radicans*.

within 0.5 - 1.0 h. The stigmas pollinated mostly would close between 15 s and 60 s, and some of them would reopen within 1 - 3 h. The reopen stigmas closed again when given more pollen grains. Stigma behavior does not show difference between treatments of self- and cross-pollination. Our results also indicate that there was no difference in pollen tube growing rate between self- and cross-pollen. Results of the stigma behavior reflection to different stigmatic pollen loads are summarized in Table 1. When no or few pollen grains stimulating, the stigmas closed and most would reopen in a short time. However, the stigma closed and only a few would reopen in a longer time when many pollen grains were deposited. All above stigmas were in the early flowers, the stigmas in the late flowers that the corollas have fallen off would not close after mechanical touch or pollen deposition.

3 Discussion

Bertin (1989) demonstrated that *C. radicans* was almost completely self-sterile following pollinations by pure self-pollen. Our study shows that the pollen tube from self-pollen grew as rapidly as the pollen tube from cross-pollen. Bertin and Sullivan (1988) also have pointed out that the ability of self pollen tubes in *C. radicans* to grow to full length as rapidly as cross pollen tubes. The interference caused by self-pollen appears to take place in the post-pollination process. This indicates that the stigma behavior could not help selecting the cross-pollen from self-pollen to avoid the negative influence that self-pollen caused on reproductive success.

Stigma closure is not limited to *C. radicans*, it is common in several families in Scrophulariales: Scrophulariaceae, Bignoniaceae, Martyniaceae (Fetscher and Kohn, 1999) and Lentibulariaceae (Newcombe, 1922). Two hypotheses have been given to explain the stigma behavior in *Mimulus aurantiacus*, a species with bilobed stigma (Fetscher and Kohn, 1999). One is that the stigma rapidly response to touch suggests that there is something to be gained by immediate response to pollinator visitation. The other is that it may prevent intrafloral selfing or reduce pollen-pistil interference. Recent study indicates that stigma closure in monkeyflower can increase pollen export: flowers with closed stigmas exported 2.8 times more pollen to recipient stigmas than did flowers with open stigmas (Fetscher *et al.*, 2002). The precise factors affecting stigma behavior in *C. radicans* remains unclear up to now.

As Bertin (1982a) mentioned, mechanical touch or pollen deposition can elicit the stigma closure in *C. radicans*. However, mechanical touch alone cannot make the stigma

Table 1 State of the stigma (open or closed, the time of closure or reopen) after pollinated by different pollen loads

Stigmatic pollen load	First closure time (s) ($n > 100$)	Reopen stigmas (%)	Reopen time (min)
>350	39 ± 11.1	10.4 (13/124)	145.8 ± 22.8 ($n=13$)
250 - 350	48.3 ± 10.9	19.8 (20/101)	122 ± 23.9 ($n=20$)
150 - 250	74.9 ± 27.6	80.4 (82/102)	86.7 ± 36.4 ($n=82$)
0 - 150	83.5 ± 26.7	94.6 (116/123)	71.9 ± 28.8 ($n=116$)

close permanently. All closed stigmas to mechanical touch would reopen, while hand pollinated pollen grains reduced significantly the possibility of stigma reopening. Moreover, detailed study shows that more than 350 pollen grains deposited on the stigmas can cause most stigmas permanent closure. We therefore suggest that the pollinated stigma secondary open may be due to the limited pollen grains deposited on the stigmas. This argument is supported by the result of Bertin (1982a), which also indicates that the stigma closure only occurs if effective pollination occurs. It is then concluded that the reopen stigmas will close permanently only when sufficient pollen grains accumulating on the stigmas. Our finding supports the statement that the closure of bilobed stigma acts as a mechanism to aid pollen receipt and capture (Thieret, 1976). Bertin (1982a) demonstrated that at least roughly 400 pollen grains should deposit on a receptive stigma to cause fruit development in *C. radicans*. Moreover, a further study by Bertin (1990a) indicated high pollen loads resulted in seeds that were more likely to germinate than seeds from low pollen loads. Therefore, sufficient stigmatic pollen loads are important to the fruit development and offspring quality in *C. radicans*. It is interesting for our results that the closure of stigma in *C. radicans* is a reflection of sufficient pollen grains deposition, which meets with the requirement of reproductive success in post-pollination process that sufficient stigmatic pollen loads are necessary for fruit development and seed quality (Bertin, 1982a; 1990a). We then suggest that as one component of plant reproductive organism, stigma character must coevolve with the whole breeding system (see also Guo and Huang, 1999; Yang *et al.*, 2002).

In summary, the bilobed stigma behavior in *C. radicans* shows no difference between self- and cross-pollen contact. In other words, our results cannot demonstrate the stigma behavior in *C. radicans* acts as an outcrossing mechanism. Combined with the studies of Bertin, our finding that the significant correlation of stigma behavior and stigmatic pollen loads suggest that the stigma behavior in *C. radicans* should be a mechanism to facilitate pollination, and then enhance the reproductive success.

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