

which bring in the 5' untranslated region cannot possibly break the stable fold. On the other hand, when the N-terminal sequence is altered as in two cases of expression of Taq polymerase^{14,15} we see expression in two different vectors. This may also indicate that the secondary structure in the mRNA after the third codon does not hinder translation initiation. Finally it may be reasonable to speculate that the wild-type Taq polymerase gene is expressed in the native strain *Thermus aquaticus* because of its thermophilic nature. At temperatures above 70°C, the secondary structure is unfolded and the translational block is released.

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Anther dimorphism, differential anther dehiscence, pollen viability and pollination success in *Caesalpinia pulcherrima* L. (Fabaceae)

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Self-compatible flowers of *Caesalpinia pulcherrima* L. (Fabaceae) exhibit dimorphism in respect of size of anthers, in their dehiscence time and filament movement. All the ten anthers borne in a flower do not anthesize simultaneously; seven small anthers dehiscence at early hours of the day (< 8.30 h) and three large anthers at mid-day. The pollen grains of small anthers were viable during early hours of the day, lose viability across time on the day of anthesis and were not viable the next day. In contrast, pollen grains of large anthers were viable till early hours of the next day. Stigma is receptive for 24 to 25 h. However, the pollen capture by stigma begins only after mid-day when the style has deflected upwards and positioned close and parallel to nectar guide. Removal of large anthers alone reduced fruit set compared to removal of small anthers alone. The adaptive advantage of this mechanism has been studied from the point of pollination success and discussed under the light of psychophilic syndrome of *Caesalpinia pulcherrima*.

TOTAL pollen production of a plant can be hierarchically divided and may be presented sequentially to reduce the risk of pollen removal by an individual pollinator during a single visit^{1–3}. This tactic results in dispersal of pollen grains to more pollinators and subsequently to several different stigma. In addition, staggered presentation of pollen will prevent weather-related deterioration on pollen. Further, scheduling of pollen presentation has been shown to effect male reproductive success⁴.

Harder and Thomson's model³ predicts that the pollen presentation to be synchronous with low pollinator visitation rates and staggered with high pollinator visitation rates. However, in contrast to predictions, *Erythronium grandiflorum* (Liliaceae) was found to stagger pollen presentation despite its low visitation rates⁵.

Staggered pollen presentation is achieved by either altering time of anthesis within an inflorescence or anther dehiscence within a flower coupled with gradual squeezing of pollen from an anther pore³. Percival^{6,7} observed that in 52 of 81 Welsh species (44.2%) anthers did not dehiscence simul-

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taneously or in the bud, and further reported that anthers dehisced over more than 1 day within a flower or inflorescence. Incidentally, it is documented that several species belonging to Fabaceae show stagger pollen presentation by having heterantherous androecium⁸ (dimorphism for anthers with vexillary aestivation differing in respect of pollen load and size, and stilar hairs⁹⁻¹³).

In *Cesalpinia pulcherrima* (Fabaceae), while seven (small) out of ten anthers dehisce during early hours of the day, the three large anthers dehisce at mid-day. The adaptive advantage of this mechanism has been studied from the point of pollination success and has been discussed in the light of plant-pollinator interaction.

The experiment was undertaken at Raichur campus of University of Agricultural Sciences, Dharwad, Raichur (16°15'N, 77°20'E; 389 m above MSL), Karnataka, India.

C. pulcherrima is a perennial woody shrub of Asiatic origin¹⁴, more precisely Indo-Malayan¹⁵. Plants bear either bright red or yellow flowers throughout the year. Flowers of *Caesalpinia* exhibit psychophilic syndrome and hence are pollinated by butterflies and moths¹⁵. The inflorescence is a compound raceme with primary, secondary, tertiary and quarternary branches emerging from a common axis and blooms in an acropetal succession. The flowers bloom between 5.30 and 6.30 h and bloomed flowers are retained on the inflorescence for well over two days with the older flowers changing their colour to deeper shades of red or yellow. The claw of upper petal in *Caesalpinia* is modified into a narrow tube which leads to the nectary¹⁵. The upper petal with nectary here has been referred to as 'nectar guide'. Nectar secretion has been observed to begin at approximately 7.30 h and continues at a relatively constant rate until mid-afternoon¹⁵.

Although self-compatible flowers of *Caesalpinia* exhibit

dimorphism in respect of size of anthers, in their dehiscence time and filament movement, they do not differ in their pollen grain size (Table 1). In both yellow and red morph flowers, seven of the ten anthers are small and the rest three are large (Table 1). The seven small anthers dehisce during early hours of the day (8.00 to 8.30 h) while they are placed parallel to nectar guide (Figure 1 a). By mid-day, both style and filaments of large anthers deflect upwards and join the filaments of short anthers (Figure 1 b). Three large anthers begin to dehisce by 12.00 h after they are positioned parallel with the nectar guide. Angles subtended by style and filaments of small and large anthers with the nectar guide have been taken as the measure of their deflection across time.

The pistil and the filaments of large anthers deflect upwards as time progresses, (Figure 2 a and b) as a result, the angle subtended between filaments of large anthers and pistil with nectar guide reduces across time. The seven small anthers which dehisce in the early hours of the day of blooming will be placed close and parallel to the nectar guide (Figure 1 a and b) while large anthers and style were placed away from the nectar guide. As the time progresses, filaments of large anthers and pistil deflect upwards such that they all come almost together and are in the same plane by 11.30 h (Figure 1 b) and remain in this plane until evening (Figure 2 a and b). The style is above all the anthers by late evening hours of the day of blooming and early hours of the next day (Figure 1 c). Stigma falls down by next day afternoon (Figure 1 d), as it loses its turgidity which is dependent upon the weather conditions.

The viability period of pollen grains was assessed, separately in both the categories of anthers, by staining the pollen grains with aniline blue and pollinating them *in vivo* at a regular interval of time across the day after their dehiscence.

Table 1. Anther size (in mm) and pollen size (value 50 μ m) in different colour morphs of *Caesalpinia pulcherrima*

Anther type	N	Mean \pm SE	t-test
Anther size (in mm)			
Yellow morph			
Large group	32	2.06 \pm 0.0097	$t = 9.87 P < 0.001$
Small group	23	1.26 \pm 0.0280	$df = 53$
Red morph			
Large group	25	2.01 \pm 0.0074	$t = 10.30 P < 0.001$
Small group	21	1.22 \pm 0.0226	$df = 44$
Pollen size (in μm)			
Yellow morph			
Large group	35	18.829 \pm 0.203	$t = 0.69 NS$
Small group	26	18.654 \pm 0.199	
Red morph			
Large group	30	19.033 \pm 0.277	$t = 8.79 P < 0.001$
Small group	23	21.870 \pm 0.238	$df = 51$

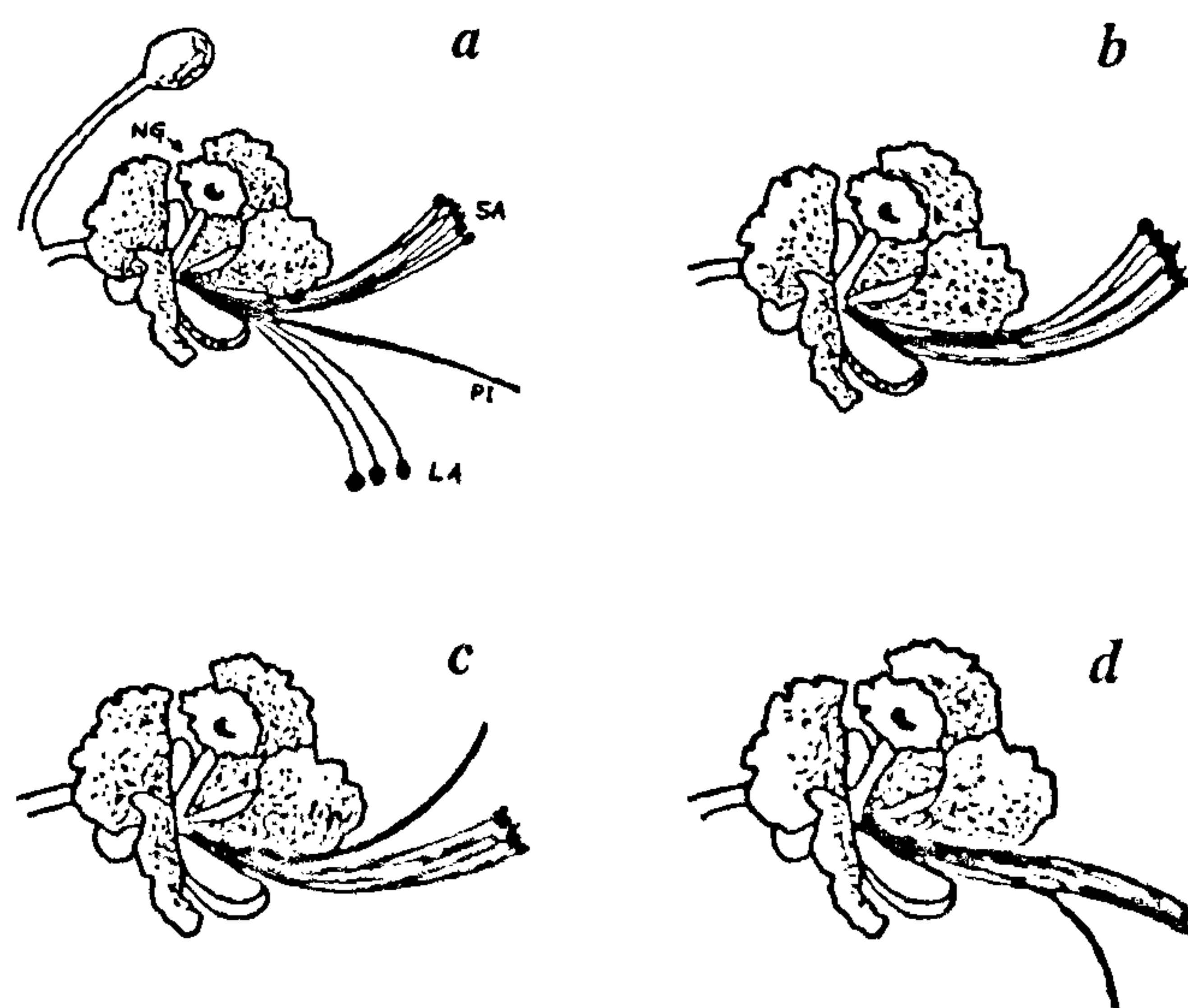


Figure 1 a-d. Natural placement of different flower parts (NG = Nectar guide; LA = large anthers; SA = small anthers) across time. a, Early hours on the day of anthesis; b, Mid-day; c, Evening hours on the day of anthesis and early hours a day later; d, Afternoon on the second day of anthesis.

RESEARCH COMMUNICATIONS

Pollen grains were germinated in 30 per cent sucrose medium to ascertain their viability¹⁶. For pollination, pollen grains from small anthers were used and stigma were pollinated at intervals of one hour from 8.30 h till 17.30 h. The stigma were pollinated with the pollen grains of large anthers between 12.30 h and 17.30 h on the day of anthesis and for both during early hours of next day between 6.00 h to 10.00 h. Only flowers with long styles were used as females for *in vivo* pollination as other category of flowers viz. those with medium, short and rudimentary styles do not set fruits (see Loksha *et al.*¹⁷).

The viability of pollen grains from small anthers, as assessed by staining, showed a general decline after 16.30 h. In both the morphs tested, pollen grains from large anthers however, were viable for a longer period of time on the day of dehiscence (i.e. 18.30 h); further, they were viable till early hours of next day as well. (Figure 3 *a* and *b*). These results were corroborated by *in vivo* pollination test (Figure 4 *a* and *b*) and by confirmation of pollen germination in 30 per cent sucrose medium. The pollination with pollen grains from

small anthers after 14.30 h decreased the fruit set; none of the flowers set to fruits which were pollinated with pollen grains of small anthers after 17.30 h. However, pollination with pollen grains of large anthers even after one day yielded about 10 per cent fruit set, suggesting the retention of pollen viability (Figure 4 *a* and *b*).

Removal of either the large or small anthers from the flowers resulted in differential fruit set (Figure 5). For example, retention of large anthers increased the fruit set compared to that of small anthers, suggesting that pollen grains of large anthers contribute substantially to self pollination (Figure 5). Data of fruit set from emasculated flowers showed that, in *Caesalpinia*, rate of cross-pollination is around 27 and 30 per cent for yellow and red morphs respectively. Retention of all anthers recorded higher (65 and 75 per cent for yellow and red morphs respectively) fruit set. This clearly indicates that self-pollination is also contributing to the ultimate fruit set of the plant (Figure 5).

From the results it is clear that the pollen grains of small anthers are viable for a shorter period (≈ 10 h), those of large anthers for a longer period (≈ 22 h). Removal of large anthers reduced fruit set compared to the removal of small anthers,

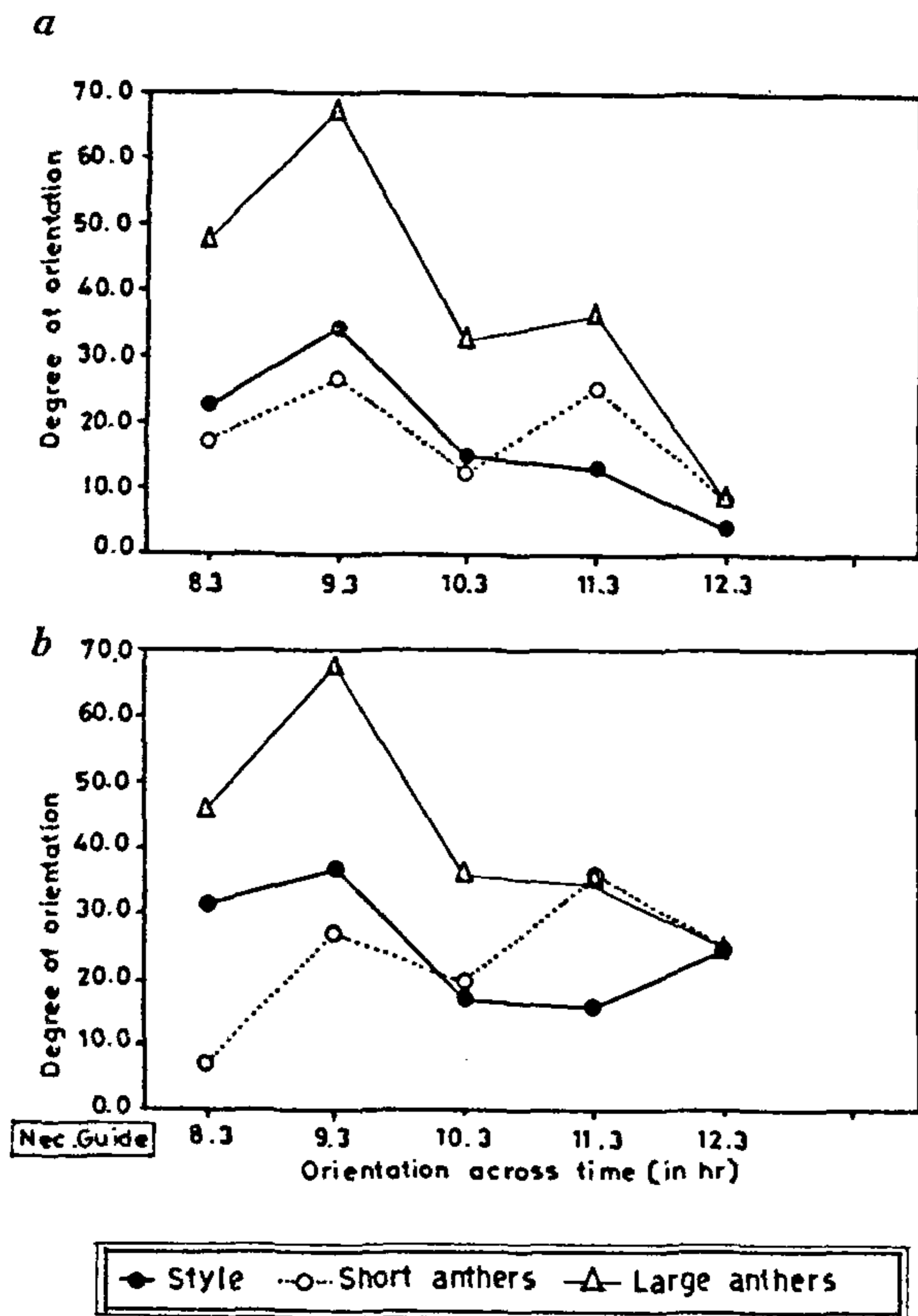


Figure 2. Angle subtended and upward deflection between anther filaments and style with nectar guide in red (*a*) and yellow (*b*) morphs of *Caesalpinia pulcherrima*.

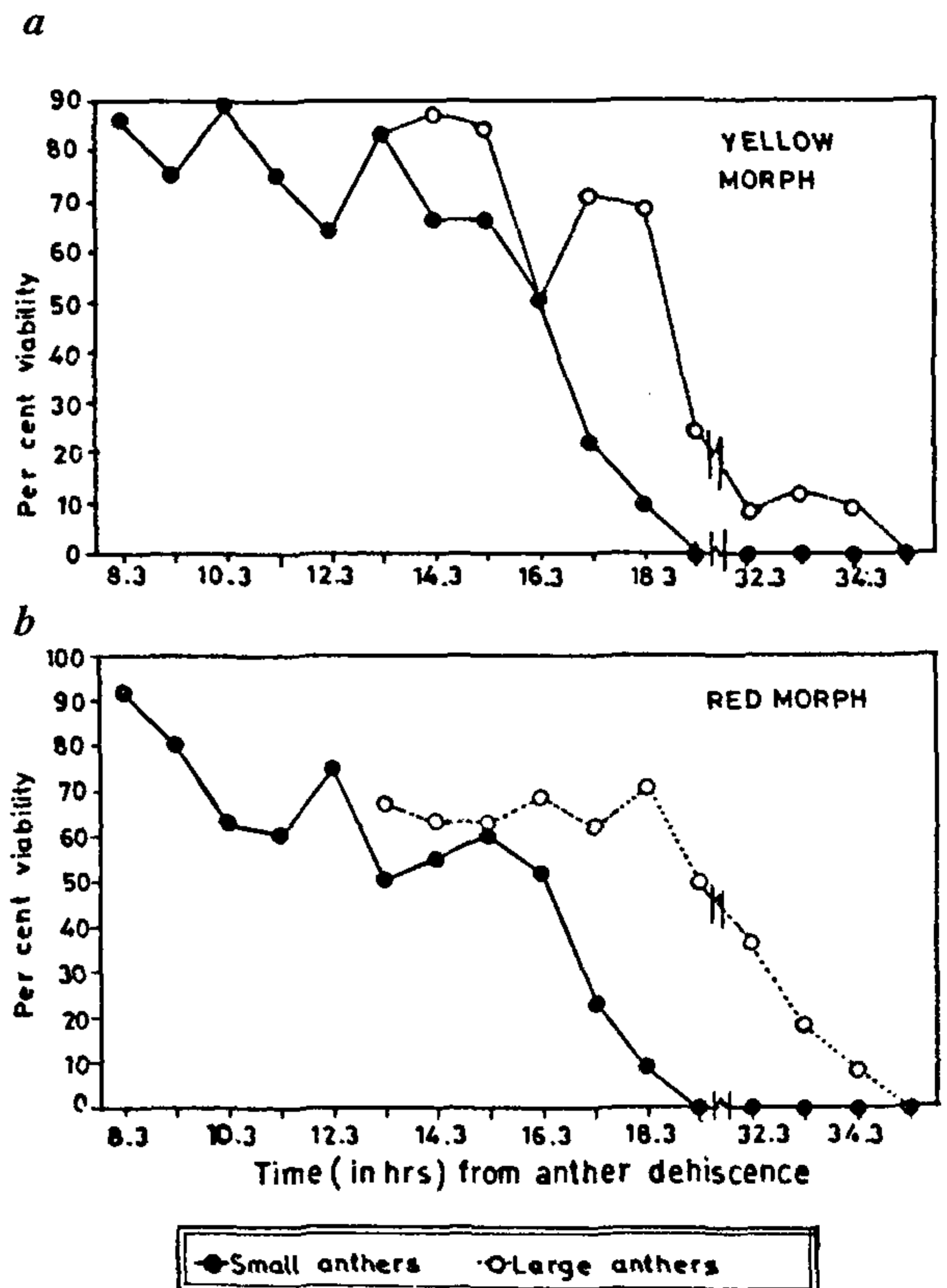


Figure 3. Pollen viability schedule in red (*a*) and yellow (*b*) morphs of *Caesalpinia pulcherrima*.

hence large anthers are the most prominent group contributing towards self and successful pollination in *C. pulcherrima*. In general, entomophilous species are considered to retain viability of pollen grains for a longer period because pollen grains must wait for pollinators¹⁸. The effective pollen donation time is extended in *Caesalpinia* by regulating anther dehiscence time so that different pollinator species such as butterflies (Papilionidae and Nymphalidae) and moths (specifically of Sphingidae) which are active during different times of the day/night are exploited for successful pollination. In fact, the stigma receptivity lasts for 24–25 h (9 am on the day of blooming till 9–10 am the next day after blooming) and hence pollen is available to receptive stigma at any time of the day. While butterflies were found to be active during early and very active during late hours of the day with almost no or reduced activity during noon (similar behaviour of butterflies has been reported by Cruden and Hermann-Parker¹⁵), the Sphingid moth was active at dusk. However, on a cloudy day both butterflies and moths were found actively foraging throughout the day.

Nectar production in *C. pulcherrima* has been shown to increase at a constant rate until mid-afternoon and it eventually ceases¹⁵. This raises the question whether or not nectar will be available in the later part of the day. (Incidentally, pollinators were over-active during evening hours indicate the nectar production to continue beyond mid-afternoon or else pollinators mostly feed on the accumulated nectar). In the absence of data on the nectar production pattern at study site and owing to the psychophilic syndrome nature of *C. pulcherrima*, the nectar production is expected to continue beyond mid-afternoon. The deleterious consequence of nonavailability or termination of nectar production in the later part of the day would be on the pollen removal (in turn cross-pollination) as activity of pollinators will reduce greatly on account of termination of nectar production because pollinators' behaviour depends on the rate and time of nectar production¹⁸. It could be that the pollinators feed on the accumulated nectar in the later part of the day.

Staggered pollen presentation is strongly dependent on pollen viability¹⁶. Intriguingly loss of viability of pollen

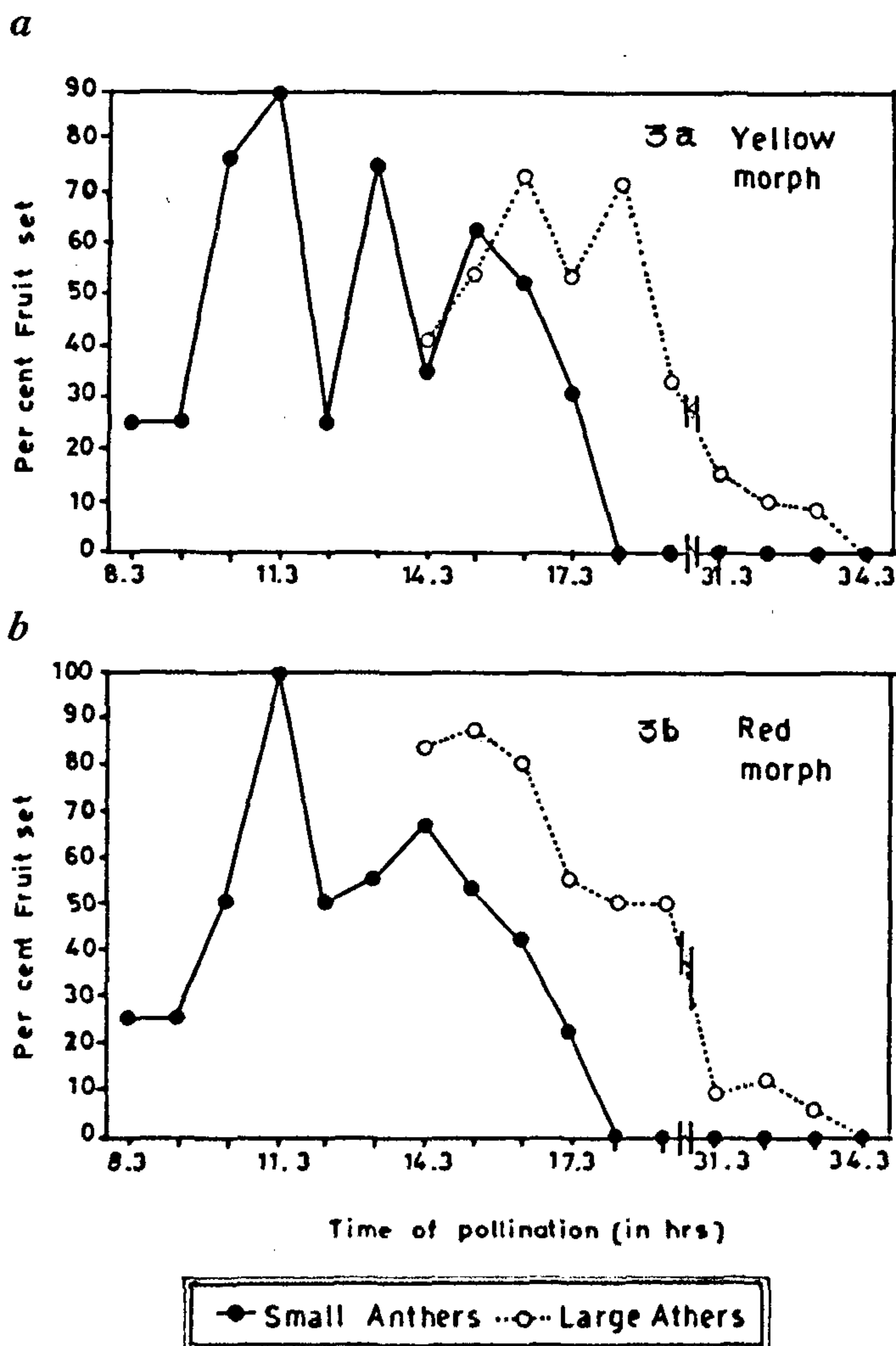


Figure 4. *In vivo* pollination and fruit set pattern in red (a) and yellow (b) morphs of *Caesalpinia pulcherrima*.

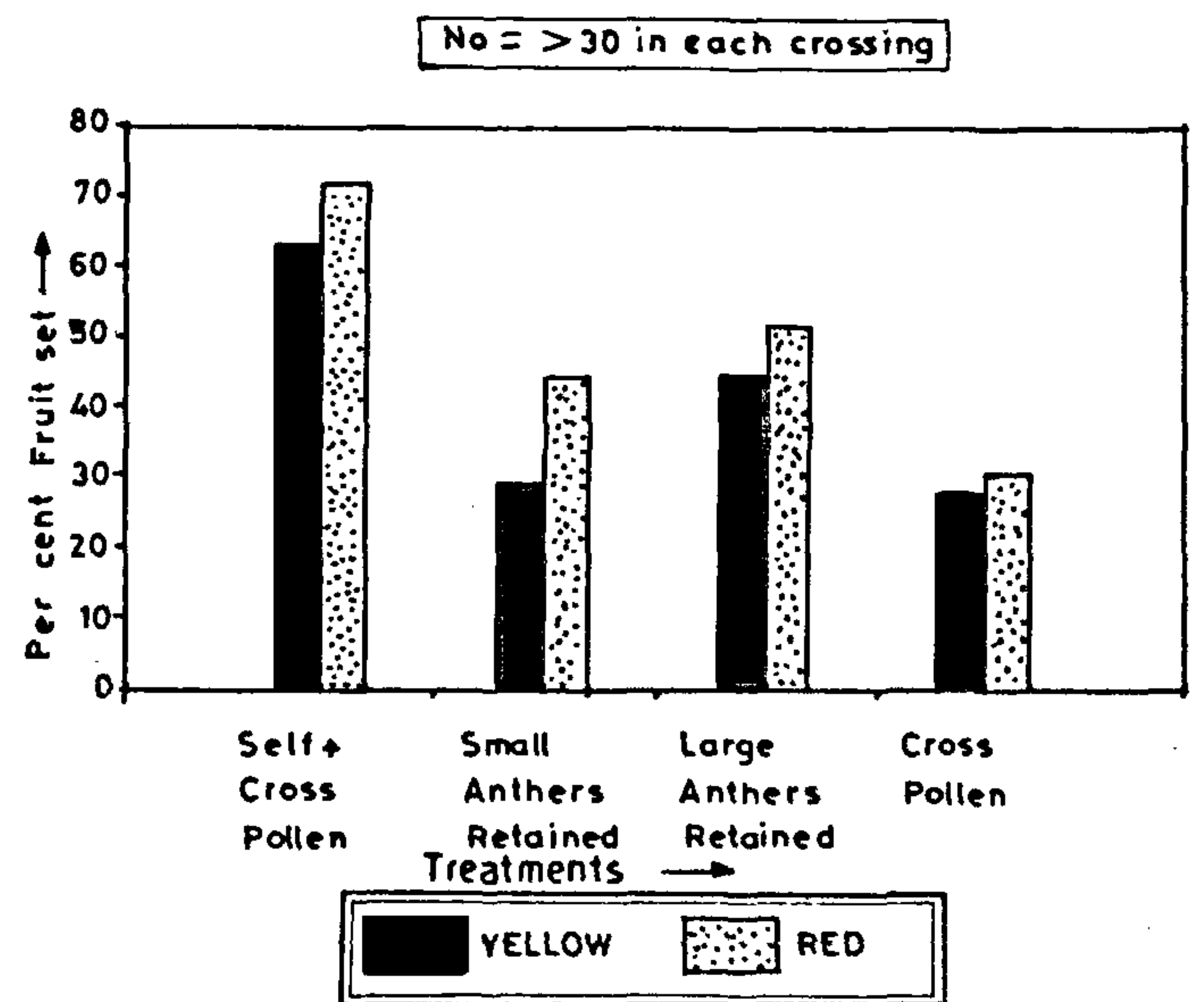


Figure 5. Effect of removal/retention of large or small anthers on fruit set in *Caesalpinia pulcherrima*.

Table 2. Progressive pollen capture by stigma of red and yellow morphs of *Caesalpinia pulcherrima*

Time in h of the day	Red		Yellow	
	N	Mean ± SD	N	Mean ± SEM
9 to 9.30 h	25	0.00 ± 0.00	25	0.00 ± 0.00
11 to 11.30 h	18	0.47 ± 0.96	19	0.42 ± 0.99
13 to 13.30 h	20	11.40 ± 14.52	18	3.22 ± 5.73
15 to 15.30 h	31	13.23 ± 15.13	30	9.35 ± 12.51
17 to 17.30 h	30	16.52 ± 14.89	29	13.39 ± 15.68

grains in a few entomophilous species across time has been reported and also has been shown to vary across species and breeding system¹⁹. Two patterns of pollen viability have been observed in entomophilous species: a rapid decline, as in *Cucurbita pepo* (Cucurbitaceae), the species with most transient pollen viability; and a gradual decline as in *Spartium junceum* L. (Leguminaceae) and *Acanthus mollis* L. (Acanthaceae) in which pollen was viable even after 72 h after the opening of the anthers¹⁹. The rapid decrease in pollen viability in *Cucurbita pepo* is related to the fact that the flowers of both sexes of this monoecious plant are accessible to insects (bees and bumble bees) for only 6 h and the pollen may be partially dehydrated²⁰. However, in *Spartium junceum* and *Acanthus mollis*, pollen grains survive, as they are protected inside the anthers, for a longer period of time²⁰. In *Erythronium grandiflorum* pollen viability decreases significantly within an hour of exposure to the air after dehiscence²¹. Pollen grains of *C. pulcherrima* are not protected but are directly exposed to weather-related factors, such as temperature and humidity. It is well documented that the pollen viability is affected by several factors other than species and breeding system, viz. individuals within a species, temperature, humidity, age and condition of pollen (bi or trinucleate)²²⁻²⁵.

Differential upward movement of filaments of large anthers, and style might represent a strategy to increase the chances of both pollen donation and pollen reception. Thus for successful pollen donation strategy, pollen should not be transported from the anthers that are positioned far away from the nectar guide and likewise for successful pollination strategy, style needs to be positioned close to the nectar guide. This was evident from the data on progressive pollen deposition on stigma which indicated that pollen count was nil when style was positioned away from the anthers during early hours of the day, while pollen deposition increased as style deflected (upward), towards nectar guide (anthers) (Table 2). No pollen deposition was observed in the flowers where style, and thence stigma, was intentionally placed away from the nectar guide.

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