Functional ecology of colour change and breeding biology in *Desmodium setigerum* (E. Mey.)

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Abstract

Floral colour change has been documented in 450 species in 78 families. It often acts as a signal to pollinators showing the flower has already been visited, improving pollinator efficiency. *Desmodium setigerum* (Leguminoseae) is a species that exhibits floral colour change and requires floral tripping to expose reproductive parts and release pollen. However, the causes of this change have previously not been studied. In this study we looked at the causes of colour change, pollinator response to flower change, flower tripping rate, visitors/pollinators and seed set in *D. setigerum*. We confirm that colour change in *D. setigerum* occurs by tripping and not pollen recognition. Both colour and morphological change caused by tripping are signals to pollinators. Tripping rate of flowers has a marked peak during the day, coinciding with the amount of open flowers and indicating a peak in pollinator activity. *D. setigerum* requires visitation to set seed which confirms the usefulness of pollinator efficiency aided by floral signalling.

INTRODUCTION

Pollination occurs as a mutualism, with plants providing a reward and pollinators providing the pollination service. It occurs in 90% of the angiosperms (Buchmann & Nabham, 1996) and Burd (1994) estimates that 62% of angiosperms are pollen limited. However, producing rewards can be costly for the plant. Nectar can comprise between 5-80% sugar (Baker & Baker, 1983) and pollen can contain up to 60% protein (Roubik, 1989). As a result, the plant does not want to waste these rewards by losing them to non-pollinating visitors or by continuing to receive visitors once the flower has been successfully pollinated.

To prevent these resource wastages, plants have evolved signalling mechanisms to signal information to their pollinators (Schaefer *et al.,* 2004). Many flowers change colour “naturally” during their life span (van Doorn, 1997). However, many are also induced to change colour by visitation and/or pollination. Nuttman & Wilmer (2003) show this inducible flower colour change in *Lupinus pilosus*. This can act as a signal to pollinators that the flower has already been visited. In turn, this allows the pollinator to forage more efficiently (van Doorn, 1997). Colour change has been documented in 450 species from 78 families (Weiss & Lamont, 1997).

Often, pollinators cannot distinguish between these signals from long distances and so some plants retain these older, colour changed flowers to act as extra long distance attractants (van Doorn, 1997). When the pollinator is close up it then can differentiate between short distance signals and may only visit the non-visited flowers.

The longevity of some flowers is also affected by pollination and is also often linked with colour change. Some species have flowers that wilt and die after pollination, but can persist for weeks without pollination (van Doorn, 1997). This again aids pollinator efficiency by causing pollinated flowers to wilt, signalling that they no longer want to be visited.

The Leguminosae exhibit 23 genera where colour change is evident (Weiss, 1995). *Desmodium setigerum* (Leguminosae) is a species that is observed to exhibit floral change colour. It also requires "tripping" by visitors to release pollen and expose the anthers and stigma (personal observations). The flowers last only one day and some may have been observed to reopen following initial colour change (personal communication: Pat Wilmer). It is unknown whether the colour change in this species is triggered by age, visitation or pollination.

The aim of this study is to examine colour change and breeding biology in *D. setigerum* by investigating the following:

- the effects of different pollen treatments on colour change and possible floral reopening of *D. setigerum*.
- the rate of colour change of *D. setigerum* in response to different pollen treatments
- the signal of colour change in *D. setigerum* to pollinators
- the morphological signal of flower tripping in *D. setigerum* to visitors
- the pattern of tripping throughout the day
- the effects of different pollen treatments on seed set of *D. setigerum*

MATERIALS AND METHODS

Study species

Desmodium setigerum E. Mey. (Leguminosae, Papillionoideae) is a herbaceous, climbing perennial. It has trifoliate leaves with silvery centre. Stems and seed pods are sticky and adhesive. Flowers are homomorphic, lilac coloured turning blue with time, and occur on terminal spikes. Flowers last for one day and require tripping by visitors to expose reproductive parts. *D. setigerum* occurs along pathways and in higher light conditions in Kibale National Park, Uganda.

Study area

This research was conducted in Kibale National Park (766 km^2) in western Uganda situated at the foothills of the Rwenzori mountains. Kibale's coordinates are 0˚13'-0˚41N and 30˚19'-30˚32E (Lwanga, 2003). The national park is classified to be moist semi-deciduous and evergreen rainforest (Wrangham *et al.,* 1994). The altitudes of the national park range from 1590 m to 990 m (Wrangham *et al*. 1994). The research site was situated in the southern part of the park near the MUBFS research station, close to the village of Kanyawara. Kanyawara's altitude is approximately 1500 m. Average annual rainfall is 1700mm (1984-1996) (Chapman *et al.* 1999). The average minimum temperature is 15 ˚C, average maximum is 20 ˚C (Chapman *et al.* 1999). In this project, *D. setigerum* was studied along the path that goes from MUBFS field station to the soccer pitch and into the regenerating forest.

Colour change experiment

To investigate floral colour change in *D. setigerum*, six pollination treatments were applied as follows:

1. self pollen (hand pollinated)
2. cross pollen (hand pollinated)
3. naturally tripped (by visitors)
4. manually tripped, no pollen (positive control)
5. not tripped (negative control)
6. heterospecific pollen (*Ipomea spp.*)

Each treatment was applied to six flowers, each flower occurring on a different inflorescence, resulting in a total of 18 replicates. The flowers in treatments 1-4 were first tripped by inserting a pin near the base of the stigma. The flowers in treatment 5 (negative control) were bagged to prevent flowers being tripped by pollinators. This experiment was carried out at three sites on three different days.

When the treatments were applied, the colour of the flowers was recorded hourly, from 10 am until 5 pm. Colour change was scored on an ordinal scale. Anther position and degree of floral openness were also recorded.

Pollinators and pollinator response to colour change

To investigate pollinators of and visitation to *D. setigerum,* observations were carried out in three sites on three different days between 10 am-5 pm. Visitors, numbers of visits, numbers of flowers visited and colour of flowers visited were recorded.

Pollinator response to morphological change

To determine whether the morphological change after tripping is a signal to pollinators, visitation to both tripped and untripped flowers was investigated. Five sites were selected and at each site an even number of flowers were mapped. Half of the flowers were manually tripped and half remained untripped. All flowers were observed over the course of time it took for the tripped flowers to completely change colour. The numbers of visits and approaches to tripped and untripped flowers were counted.

Tripping rate

To determine the rate at which *D. setigerum* flowers were visited, two transect walks were carried out. Every hour, from 8 am to 6 pm, all receptive tripped and untripped flowers on the transect line were counted.

Seed set experiment

To examine seed set of *D. setigerum*, 5 pollination treatments were applied (1-5 as above) in 9 sites. Each treatment was applied to 6 flowers, each flower occurring on a different inflorescence, resulting in a total of 54 replicates. The treated flowers were left for 5 days. The seeds were then collected and counted back in the lab.

Statistical analysis

For all the statistical analyses the statistical package MINITAB was used. Where data was normally distributed ANOVA's were carried out followed up by a Tukey post-hoc test. For data that was not normally distributed a Kruskal-Wallis test was used. In the pollinator response to morphological change experiment a Chi-square test was carried out. To test for correlations in the colour change experiment a Spearman rank test was used.

RESULTS

Colour change

The lifespan of *D. setigerum* flowers is one day and all flowers were observed to change colour throughout the day. Colour change differs between tripped and untripped flowers. The stages of colour change of tripped flowers was as follows:

1. Lilac – an open flower not yet visited,
2. Lilac visited – an open, tripped flower
3. Lilac pale – first signs of colour change
4. White over blue/lilac – white flag and blue/lilac keel
5. White over blue – white flag over blue keel

Stage 5 was the final stage. Some flowers were recorded as blue as the flag was bent back but this was most likely due to morphological damage. In stage 1 the flowers are fully open with anthers visible in centre. By stage 5 the flowers are fully closed with anthers under flag (Table 1). Flower colour is positively correlated with both anther position (Spearman Rank Correlation: Ps = 0.741, $p<0.001$) and angle of flag to keel (Spearman Rank Correlation: Ps = 0.766, $p<0.001$).

Table 1. Stages of colour change, anther and stigma position and angle of flag to keel.

Stage	Colour	Description	Anthers and stigma	Angle of flag to keel
1	Lilac	Lilac open flower not visited	Under untripped keel	>120 degrees
2	Lilac visited	Open, tripped flower	Tripped in middle	>120 degrees
3	Lilac pale	First sign of pollen change	Tripped in middle	>100 degrees
4	White over blue/lilac	White flag over blue/lilac keel	Hidden under lowering flag	80-90 degrees
5	White over blue	White flag over blue keel	Hidden under lowered flag	80 degrees or less

The stages of colour change in untripped flowers differ from tripped as follows:

1. Lilac – an open flower not yet visited
2. Blue over lilac – an open flower with blue flag over lilac keel
3. White over blue – a white flag over a blue keel.

Stage 3 was not recorded in our observation period but was noted the following morning in all cases.

Fig. 1 shows the percentage of all the colour stages of both tripped and untripped flowers as they occur during the day.

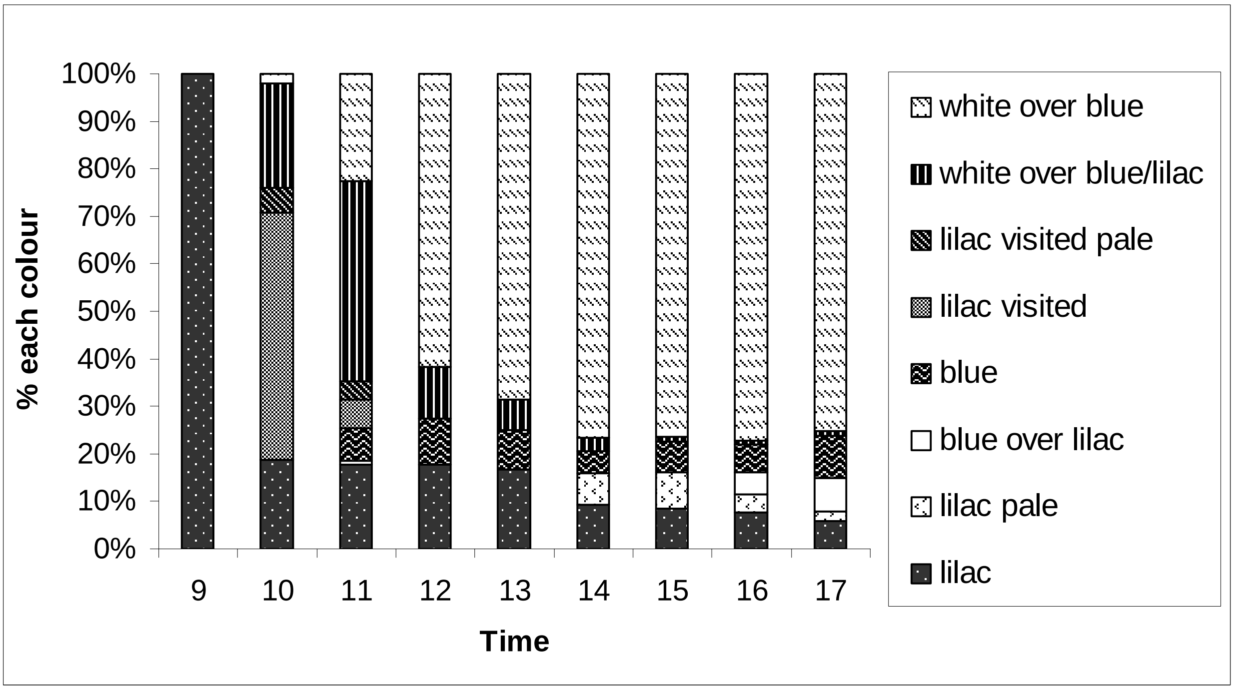

Figure 1. The percentage of flowers in each colour category over time in *D. setigerum*.

In order to compare the different rates of colour change among treatments, the time taken to change from stage 1 (lilac) to stage 5 (white over blue) was calculated. As the flowers in the untripped treatment never reached the final stage during our observation period, we assumed 6 pm as their end point. However, it is likely these flowers took longer to complete their colour change.

The time taken to complete colour change varied significantly among pollen treatments (One-way ANOVA: $F_{5,107}$=428.83, p<0.001). No significant difference was found among the first five treatments (tripped) but untripped flowers took significantly longer to complete their colour change (Tukey post-hoc test) (Fig. 2). No flowers were observed to reopen following initial colour change.

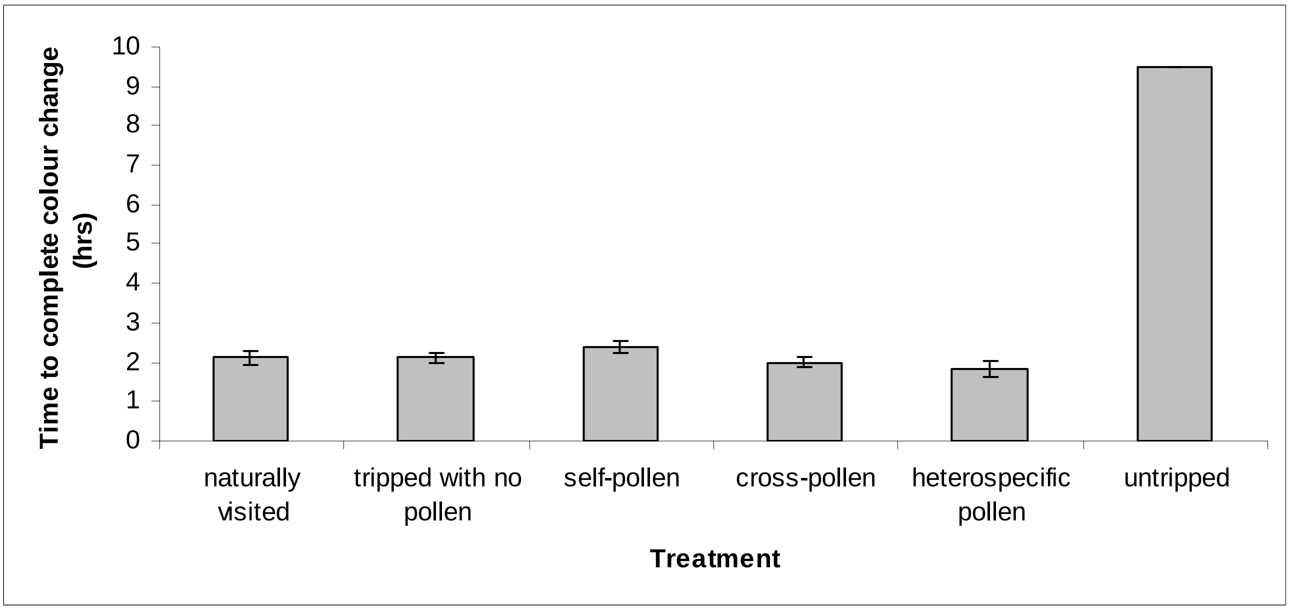

Fig. 2 The mean time to complete colour change from "lilac" to "blue over white" in *D. setigerum* flowers for each pollen treatment.

Pollinators and pollinator response to colour change

A wide variety of visitors were seen to visit *D. setigerum* flowers. Bees were the only visitors capable of tripping the flowers. Fifteen bee species were caught tripping *D. setigerum* flowers and a number of others were also observed.

There was a huge range in size of bee capable of triggering the tripping mechanism. Observed tripping visitors ranged from small bees (<0.5 cm) to large *Xylocopa spp*. (up to 3 cm). Although very uncommon, some secondary visitors were observed. One species of bee was seen to "rob" by piercing the underside of an untripped keel and collect pollen.

From visitation observations we noted that visits occurred almost solely to lilac flowers (Fig. 3). Visits to blue/white flowers were very uncommon (6/113 visits) and were mostly by non-bee species.

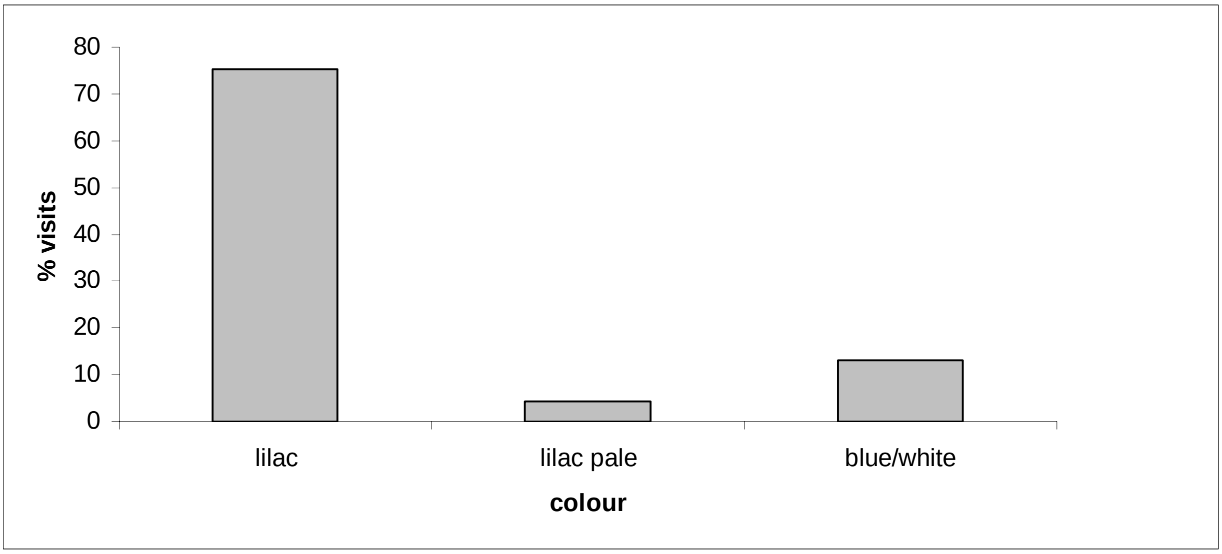

Fig. 3 The percentage of visits to *D. setigerum* flowers in each of three colour categories. "Lilac" category contains colour stages 1-2, "Lilac pale" contains stage 3 and blue/white contains stages 4-5.

Pollinator response to morphological change

Although visitors appeared to have a preference for lilac flowers, there was a marked difference in their response to whether the flowers were tripped or untripped. Untripped flowers received significantly more visits than tripped flowers. However, tripped flowers were approached more often than tripped flowers (χ^2 =104.56, df=1, p<0.001).The number of approaches to tripped flowers was similar to the number of visits to untripped flowers, but visitors distinguished flower status close up and did not actually visit (Fig. 4).

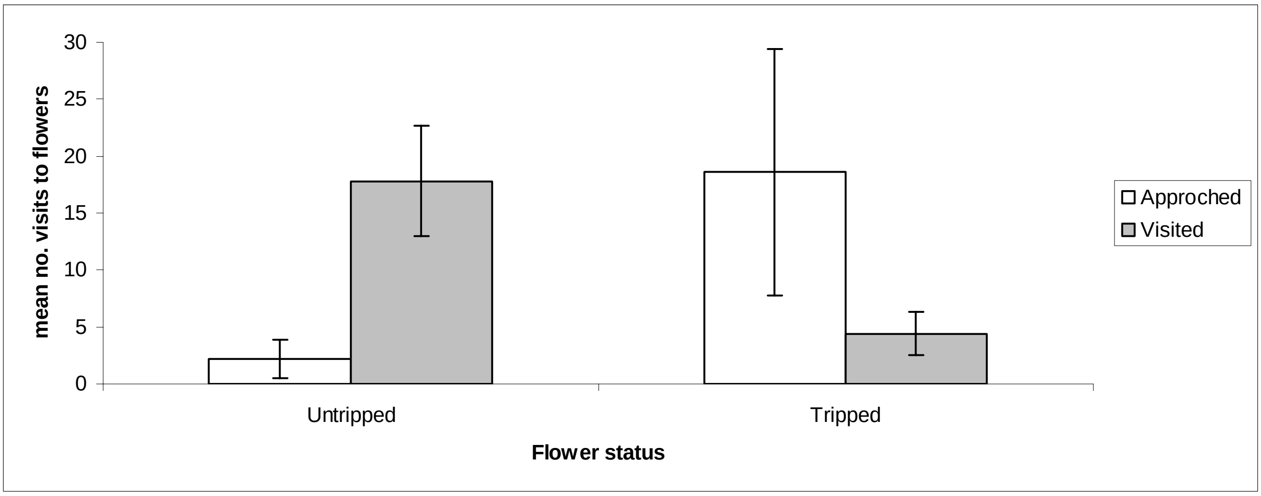

Fig. 4. The preference of visitors for visiting untripped flowers. "Approached" signifies an approach without landing. "Visited" signifies a visit.

Tripping rate

During the day there was a peak in number of tripped flowers. Early in the morning the majority of flowers were closed. By 11am most buds had opened. By this time visitors appeared most active as the highest number of flowers were tripped (Fig. 5). Tripping visitation appears to peak between 9 am to 12 pm.

Flowers that were tripped and still open with some degree of lilac were recorded as tripped. As a result, the number of tripped flowers decreased during the day as flowers completed their colour change.

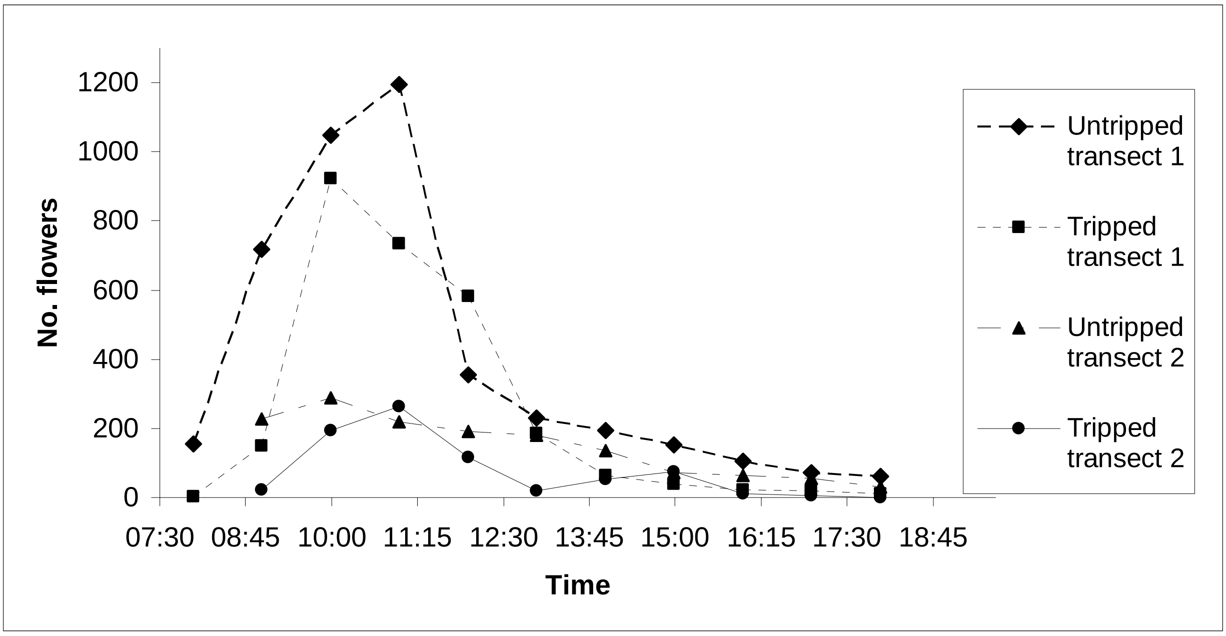

Fig. 5 The number of tripped and untripped flowers from 8am to 6pm on 2 transect lines.

Seed set

Seed set varied significantly among the pollen treatments applied (Kruskal-Wallis, $H=43.52$, $df=4$, $p<0.001$). It appears that *D. setigerum* needs some form of pollen application to the stigma after tripping to set seed, as the seed set in “tripped with no pollen” and “untripped control” was almost zero (Fig. 6).

A large number of flowers treated in this experiment had fallen off their petiole when seeds were collected. This occurred in almost all the “tripped with no pollen” and “untripped control” replicates where we predicted little to no seed set, as well as many of the others. As a result the assumption was made that where the flowers were lost no seed set would have occurred. However some of these flowers may also have been lost due to physical damage (heavy rain etc.).

Fig. 6 Seed set under five different pollen treatments. "naturally tripped" n=56, "tripped with no pollen" n=53, "self-pollen" n=49, "cross-pollen" n=49, "untripped" n=56.

CONCLUSION AND DISCUSSION

This study confirms that colour change in *D. setigerum* occurs by tripping and not pollen recognition. Colour and morphological change are signals to pollinators. Tripping rate of flowers has a marked peak coinciding with the amount of open flowers indicating a peak in pollinator activity. *D. setigerum* requires visitation to set seed and although can set seed with self pollen, does not self pollinate.

Colour change in *D. setigerum* is an induced reaction. As in other documented species (Nuttman & Willmer, 2003), colour change is induced by visitation. In *D. setigerum*, it is the act of tripping and not pollen deposition that causes the floral colour change to accelerate. Therefore the plant may change colour even if not pollinated as the tripping mechanism and not pollen recognition stimulates colour change. However, tripping without some degree of pollen deposition is unlikely as tripping without visitation does not occur. Colour change is also induced at a much slower rate by floral ageing. As these flowers last for only one day, colour change without tripping occurs near the end of the lifespan of the flower.

The rapid colour change in *D. setigerum* flowers allows the plant to signal to its pollinators. All flowers begin the day with an equal attractiveness as they are all lilac. During the day, those flowers that were tripped change colour and become less attractive, increasing the chances of their unvisited neighbours to be pollinated.

Van Doorn (1997) suggests induced floral colour change does not exist in flowers that last only one day. He states that such rapid change would not increase effectiveness of pollination as the lifespan of the flower is already so short. In *D. setigerum*, however, we find rapid colour change in flowers that last only one day. Colour change in most flowers is a signal to pollinators that pollination has occurred but may also be to reduce maintenance cost of the flowers (van Doorn, 1997). In *D. setigerum*, colour change must increase effectiveness of pollination as it cannot reduce maintenance cost of the flowers as they already live for such a short period.

Before colour change occurs, floral morphology also acts as a signal to pollinators. From a long distance, visitors appear unable to distinguish between tripped and untripped flowers and the larger display attracts more visitors. From a short distance, the morphological signal of flower tripping is recognised and tripped flowers are rarely revisited. The visitor would approach to within 1cm of a tripped flower without consequently landing on the flower. This long distance attraction with different signals close up in commonly found in plants that change colour, increasing pollinators attraction but without losing pollinator efficiency and reducing geitonogamy (van Doorn, 1997, Weiss, 1995).

This dual signalling in *D. setigerum* creates an effective overall signal to visitors. Very few visitors were recorded to visit flowers displaying either of these signals. This dual signalling must increase pollinator efficiency, as suggested by the extremely small number of untripped flowers that remain at the end of the day.

The signalling mechanisms in this species may be necessary as the plant requires visitation to set seed. Untripped flowers usually do not set seed, nor do tripped flowers without a visitor showing the plant does not self pollinate. This is due to the fact that pollen is released in an explosive fashion only after tripping. The explosive pollen release in this species prevents self pollination as pollen is dispersed away from the flower. *D. setigerum* is, however, capable of setting seed equally with self and non-self pollen showing it does not have a homomorphic self-incompatibility mechanism, but as pollen explosion requires tripping self pollination would rarely occur.

This species is visited and tripped by a large range of bee species. These visitors have a huge range in body size, from very small bees (<0.5 cm) to large *Xylocopa spp* (3 cm). There also appears to be a large range of tongue length. It is unusual for a plant species with a specialised tripping mechanism to have such a wide variety of visitors. All species appeared to respond equally to the signals of the flowers bur perhaps not all these species are equally effective pollinators. In this case the specialised tripping mechanism would not be effective in excluding non-pollinators. The plant would still give out signals that it has been pollinated but would only be tripped and not have received any pollen.

For further studies, it would be interesting to investigate which pollinators are the most effective. Also, further study on the pollinator response to the signals of *D. setigerum* flowers would be beneficial. This could investigate whether different pollinators respond differently on a more sensitive scale to these signals. Secondly, this could gain insight into how pollinator efficiency and signalling mechanisms are related.

This study has provided baseline ecological information on the signalling and breeding biology of *D. setigerum*. This species, with short floral longevity, has a surprisingly complex set of floral signalling mechanisms to visitors. These signals appear to be effective in increasing the reproductive success of the plant. Further studies on other species would be interesting to see if this is a common or indeed unique occurrence.

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