

PHOTOPERIOD REGULATES CAPE SUNDEW (*DROSERA CAPENSIS*)
GLAND SECRETION AND LEAF DEVELOPMENT

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Abstract: Cape Sundew (*Drosera capensis*), a carnivorous plant that catches flies with sticky mucus, has attracted great interest among botanists and horticulture hobbyists since the Darwin era. But little is known about how this carnivorous plant regulates morphogenesis and organ formation to accommodate environmental changes. In this article we present the relationship between gland secretion of Cape Sundew and photoperiod utilizing various physiological and morphological methods. We show that Cape Sundew grows faster and secretes more mucus under long days than under short days. Under long days leaf length and the blade\petiole ratio increases, leading to increased fly catching capacities. More importantly, in the short term, the rhythm of photoperiod causes Cape Sundew to secrete mucus independent of photo intensity.

Introduction

As one of the most special plant groups, carnivorous plants perform photosynthesis and feed on insects and some large carnivorous plants even prey on birds and small mammals. Darwin believed that a carnivorous plant was one of the most astonishing phenomena in the world (Darwin 1875; Ellison & Gotelli 2009). Carnivorous plants are represented by more than 600 species belonging to 20 genera (Ellison & Gotelli 2001; McPherson 2010). Before the 19th century, the majority of naturalists and gardeners believed that “There were no plants that could eat animals because they had no mouth to eat or stomach to digest” (McPherson 2010). Darwin and his son were strongly opposed by people because nobody believed the existence of carnivorous plants. Despite the opposition, Darwin and his son fully verified by “Darwin-style research” that *Drosera rotundifolia* could catch and digest insects. Carnivorous plants can be divided into three insect-catching mechanisms: 1) catches insects by quick movement (*Dionaea muscipula*), 2) by mucus (*Drosera*), 3) by pit-fall traps (*Nepenthes*). *Drosera* is the most widely distributed genus with the largest number of species and can survive not only in the Asian, European, and American wet lands, but also in the seasonal drought regions in the Southern Hemisphere. Despite numerous species and huge differences in surrounding environment, most *Drosera* live in a non-nutritive acid soil environment where the organic substances are slowly decomposed. *Drosera* catch insects by mucus secretion. When a *Drosera* catches insects with its extremely sticky mucus, its leaf will become bent to wrap the insects and form an external stomach which secretes digestive liquid. In this process, the phytohormone jasmonic acid (JA) plays a major role (Nakamura *et al.* 2013). There are also species which catch insects by both quick movement and mucus. For instance, the tentacle of *Drosera glanduligera*, a sundew from southern Austra-

lia, swiftly catapults prey onto adjacent sticky mucus-tentacles and then digest it (Poppinga *et al.* 2012).

The leaves of carnivorous plants not only perform photosynthesis, but also catch insects. These specialized leaves, however, have low photosynthetic efficiency compared with normal leaves, which is believed to be an adaptation to carnivorous behaviors. A “cost-benefit” model was presented by Pavlovic (2010) to explain this phenomenon. Plants need to predict seasonal changes by perceiving photoperiod signals, and then adjust their growth and development process accordingly (Putterill *et al.* 1995; Kobayashi & Weigel 2007; Harmer 2009). In this paper we show that photoperiod regulates leaf growth and mucus secretion in *Drosera capensis*, providing new insights into our understanding of carnivorous plants.

Method and Materials

Plant culture

Drosera capensis were transplanted into moist soil and covered with moss. For photoperiod experiments, plants were grown in chambers under either long day (LD:16/8 h; light intensity 3500 Lux) or short day (SD:8/16 h; light intensity 3500 Lux). For different light intensity experiments “LD 1/2” was 1800 Lux, and “SD 2 times” was about 7460 Lux. The greenhouse humidity was 50-70% and the temperature was kept at 25°C.

For each treatment, lengths of petiole and blade were measured on more than 3 leaves every week (accurate to 1 mm) and blade/petiole ratio was calculated. In addition, a dissecting microscope (Moticam 2306) was used to observe gland secretion. Both the observation and the measurement lasted more than two months.

Scanning electron microscopy (SEM) method

Fixation: Samples were soaked in FAA fixative (100% alcohol: 50 ml; 37-40% formaldehyde: 10 ml; acetic acid: 5 ml), vacuumed gently until the samples sank to the bottom, fixed for 12-24 hours, and the solution was changed once. Gradient dehydration: 50% ethanol 20-30 min; 70% ethanol 20-30 min; 90% ethanol 20-30 min; 100% ethanol twice, 20-30 min each. Critical point drying: 6 cycles. Spraying: 15 nm thickness, 90 seconds. Finally, electronic microscope photographs.

Results

Droseraceae leaf morphology

Drosera belongs to the plant family *Droseraceae*. There are great leaf morphological differences among different species. *Drosera spatulata* (Fig. 1B), has spoon-shaped leaves with no obvious boundary between petiole and blade, and its glands are in the distal part. *Drosera pulchella* (Fig. 1C) has a round blade with glands in the distal end, the proximal part is flat without glands. Leaves of *Drosera aliciae* (Fig. 1D) are ligulate-shaped without obvious blade and petiole boundaries, and has glands covering all of the leaf. *Drosera capensis* (Fig. 1A) leaf has an obvious boundary, which was selected as the main experimental material because they were easy to grow and reproduce. The *Drosera capensis* leaf can be divided into two domains (dominant): The proximal part of leaf petiole and the abaxial end blade part (Fig. 1A and E). Glands only grow in the blade and not in the petiole. As shown by SEM (scanning electron microscopy), a gland can be divided into a globular head and a rod-shaped base, which is connected to the surface of the



Figure 1: The leaf morphology of *Drosera*. (A) *Drosera capensis*, white arrow indicates petiole, black arrow indicates blade; (B) *Drosera spatulata*; (C) *Drosera pulchella*; (D) *Drosera aliciae*; (E) *Drosera capensis*; (F-G) *Drosera capensis* inflorescence.

blade (Fig. 3A and D). After full expansion of the leaves, glands begin to secrete mucus (Figs. 1E and 2B) and have the ability to capture insects. After a period of vegetative growth, the plant starts flowering. Their inflorescences are curled and gradually open during the elongation process. This type of bolting could keep flower and pollinators away from the dangerous sticky leaves (Fig. 1F-G).

Long day environment activates gland secretion

Exposed under the LD condition for one week, *Drosera capensis* grew faster than those under SD (Fig. 2A). Leaves were long and flat, which favor catching insects. The gland secreted much mucus covering the head, which was extremely sticky (Fig. 2B). Under SD, leaves were curly and small. There was no or very little mucus on the gland head (Figs. 2C and 3D-F). However, the structure of gland didn't show any significant morphologic difference between LD and SD, except SD gland's diameter is slightly smaller than

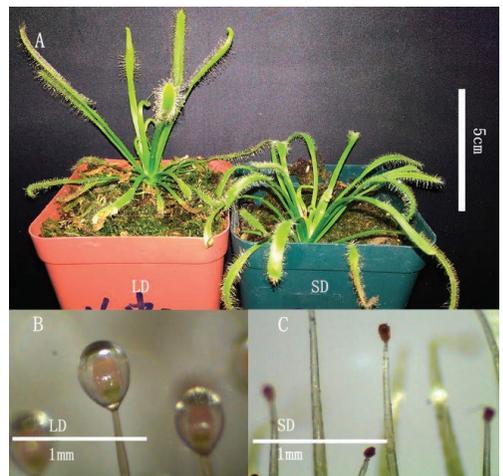


Figure 2: *Drosera capensis* phenotypes (A) under LD and SD. Gland mucus secretion LD (B) and SD (C).

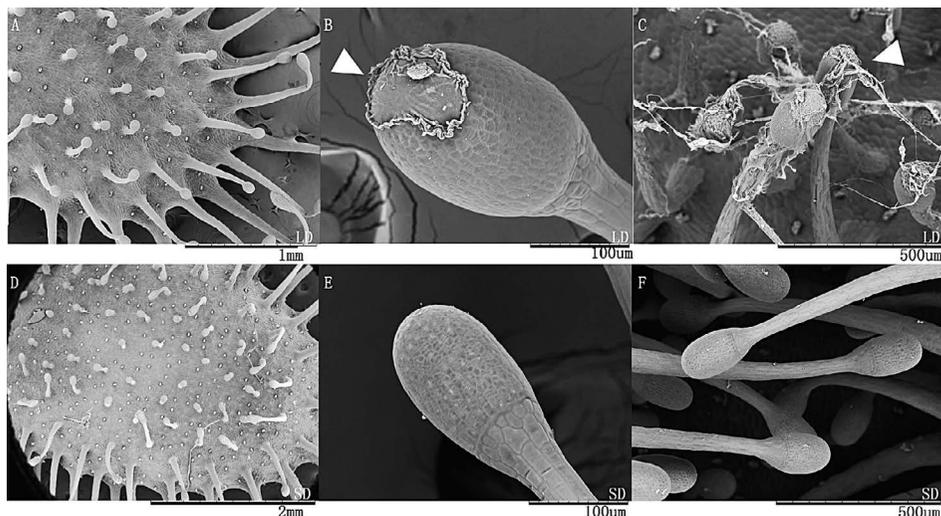


Figure 3: SEM of *Drosera capensis* gland on LD and SD. Leaf on LD (A); the head secreted mucus (white arrow) (B-C). On SD (D), the gland had no significant morphologic difference compared with LD, but there was no mucus on the glands (E-F). The scale is indicated by the black bar.

LD (Fig. 3B and E). The gland on SD still has the secretion function because it secreted mucus again after it was put back to LD for one week.

LD increases the ratio of blade and petiole

The day length not only affects gland secretion in short term, but also could affect leaf morphology in long term. The *Drosera capensis*, which leaf has a ratio of blade and petiole close to 1:1, were put into LD and SD respectively. After two weeks, the blade gradually grew longer than the petiole under LD. However, the plant moved to SD has a longer petiole and shorter blade compared with the LD plant (Fig. 4A), but the full length of the leaf had no statistical difference up to six weeks (Fig. 4B). The newly developed leaves were also measured after 5 weeks. The new leaves on LD have higher blade/petiole ratio and longer full leaf length than SD (Fig. 4C and D).

Rhythm of photoperiod is the main factor affecting gland secretion in the short term in *Drosera capensis*

Different light conditions could cause rhythm difference as well as energy difference. Under LD condition *Drosera capensis* gets more solar energy, which could affect plant development in many ways. To further investigate which aspect of photoperiod plays a more important role, a series of experiments were performed. *Drosera capensis* plants were grown for 6 weeks under LD and SD respectively. Then the SD plants were moved back to LD with full illumination intensity (Fig. 5C and H) and SD with twofold illumination intensity (SD + 2X light) (Fig. 5E and J). The LD plants were also moved to LD with half-illumination intensity (LD + 1/2 light) (Fig. 5D and I). After 1 week, plants grown under LD (Fig. 5A and F), LD + 1/2 light (Fig. 5D and I), and from SD to LD (Fig. 5C and H) were able to secrete mucus. The plants grew under SD (Fig. 5B and G) and SD + 2X light (Fig. 5E and J) still haven't had any mucus. All of the results above show that it was the rhythm of photoperiod rather than the quantity of solar energy that regulates gland secretion in the short term.

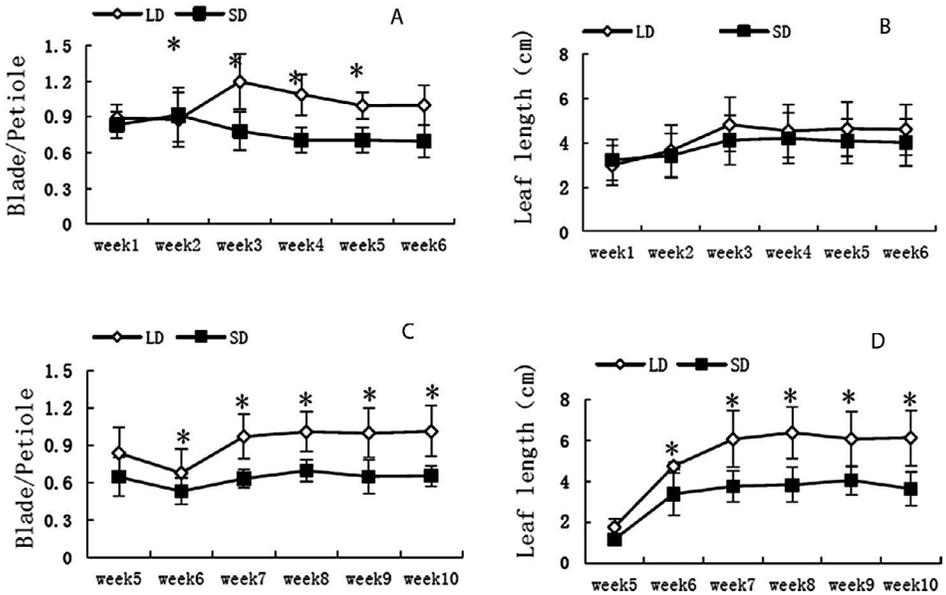


Figure 4: Different photoperiods affect the leaf blade/petiole ratio and leaf length. (A) *Drosera capensis* leaf blade/petiole ratio on LD and SD. (B) Leaf length on LD and SD (number of leaves, N=16-20). (C) The blade/petiole ratio of newly developed leaf on LD and SD. (D) The newly developed leaves length on LD and SD. (* T-test, P<0.05. number of leaves, N=18-38).

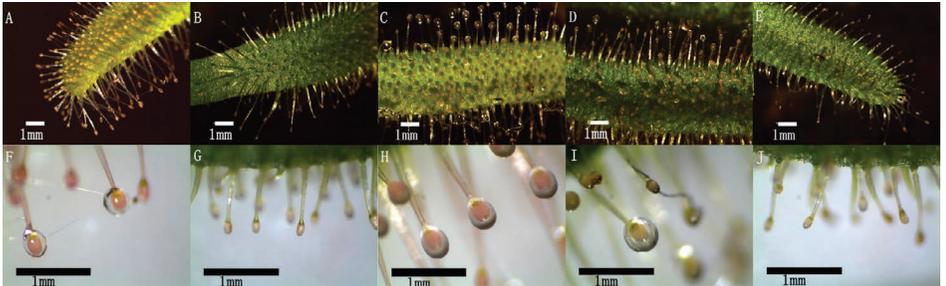


Figure 5: Photoperiod regulates *Drosera capensis* glands secretion independent of illumination intensity. (A & F) on LD; (B & G) on SD; (C & H) on SD moved to LD 1 week; (D & I) on LD moved to LD+1/2 light 1 week; (E & J) on SD moved to SD + 2x light 1 week.

Discussion

In this paper with different photoperiod and illumination intensity treatments, combined with morphological analysis, we demonstrated that sundew (carnivorous plant) adapts to seasonal changes in different photoperiod environments by regulating the quantity of mucus secretion and adjusting the proportion of insect-catching (leaf blade) and non-insect-catching (leaf petiole) parts. Compared with LD it was obvious that the secretion function of glands under SD was decreased, and leaf blade elongation was also inhibited. After switching from SD environment to LD environment, secretion ability can be fully recovered which indicated that the morphological structure and func-

tion of secretion were normal in both processes of growth. In LD condition, when illumination intensity was decreased by half, the quantity of mucus secretion had no significant change. Similarly, in SD condition, even when illumination intensity was increased by two times, the secreting gland cannot secrete mucus in the short term. The experimental results above indicated that the secretion ability of the secreting gland of sundew was mainly influenced by the rhythm changes of different photoperiods. In addition to the short term effect of mucus secretion, growing in SD environment for a long time, the sundew had obvious phenotypes such as short and curled leaf. Moreover, the proportion of leaf blade was less than for the LD plant.

It's crucial for carnivorous plants whether insects can be caught. Mostly, carnivorous plants grow in the environment where there is a lack of nutrition and cannot meet the demand of absorbing elements such as N and P by the root. Therefore, the carnivorous plants catch insects instead of root nutrient absorption to maintain growth. The same as normal plants, carnivorous plants also forecast and feel seasonal changes through rhythm changes of photoperiod. The glandular secretion of leaf mucus is the only weapon for sundews to catch insects. In an environment where seasonal changes are significant, the quantity of insects is usually rare in winter. At this time, it's not worth consuming too much energy to grow insect-catching organs and secreting a lot of mucus. However, in spring and summer there are plenty of insects in the environment and sundews can secrete a lot of mucus at this time to catch a lot of insects to meet the demand of fast growth. In addition to sundews which can regulate mucus secretion initiatively, pitcher plants were also reported to have passive regulation. When dew drops gather on the peristome of pitcher plants (*Nepenthes rafflesiana*) it will become unusually smooth, which makes it easier for ants to fall and increases the opportunity to catch insects. However, the existence of dew drops depends on external moisture variation (Bauer *et al.* 2015). As for Venus flytrap (*Dionaea muscipula*), it also has the ability to adapt to seasonal changes. The Venus flytrap leaf grows along the ground and the petiole of expands in winter, while the trapping lobe which is in charge of catching insects is smaller and loses the ability to close, which makes it hard to catch insects (unpublished observation).

Compared with normal plants, carnivorous plants need to prioritize between photosynthesis and getting nitrogen resources. Photoperiod regulates the mucus secretion and leaf morphogenesis of sundews, which provides a new research direction to study this issue. Since Darwin's day, carnivorous plants have been studied extensively. The results obtained by modern biology will help people to further understand carnivorous plants.

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