Journal of Modern Crop Science (2023)
Clausius Scientific Press, Canada

DOI: 10.23977/jmcs.2023.020109
ISSN 2616-2075 Vol. 2 Num. 1

The closure regularity of the touch-sensitive stigma of Mazus miquelii under artificial stimulation

Yitong Yang

Hongyi College, Wuhan University, Luojia Mountain, Wuhan, Hubei, 430072, China

Keywords: Stolonifera, Touch-sensitive stigma, Population, Floral characteristics, Inflorescence

Abstract: A type of stigma exists in angiosperms that closes shortly after being touched by pollinators. At present, the research on touch-sensitive stigma mainly focuses on the ecology of pollination, while the dynamic change of stigma closure is rarely studied. The larger the stigma, the faster the temporary closing speed. The dynamic changes of the stigma may be related to its internal physiological mechanism. Similar to the closing of the leaf trap of Venus flytrap, the closing movement of the touch-sensitive stigma was found to be closely related to action potentials. The study of the movement law of Venus flytrap may provide guidance for the biological law of touch-sensitive stigma. In this experiment, two populations of Rhizoma stolonifera in Wuhan Botanical Garden were used as the experimental objects, which were planted in a laboratory with no pollinators and good light and ventilation. The open stigma was selected for uniform mechanical stimulation, and the closing process of the stigma was recorded. Combined with the characteristics of the flower and the number of flowers on the inflorescence, the law of the temporary closing time of the stigma was summarized, and the movement pattern of the closing process was analyzed. The experimental results show that there are significant differences in the temporary closure time of stigma in different populations; the temporary closure time on the same inflorescence has a decreasing trend in the order from bottom to top; and there were also different movement patterns, and the differences in movement patterns were not significantly correlated with population, flower characteristics, or flower position on the inflorescence. This research is a preliminary attempt on the movement law of the touch-sensitive stigma. Through video recording, the speed can be accurately measured by judging the number of frames in the first, middle and last three time periods. However, in the later stage, high-speed cameras and corresponding image recognition software are needed to accurately extract information about the movement of the stigma.

1. Introduction

1.1 Distribution and general rules of touch-sensitive stigma

The closure of touch sensitive stigmas in flowering plants has been one of the hot issues in pollination ecology and evolutionary biology. The phenomenon that the multi lobed stigmas of plant flowers close rapidly after pollinator contact mainly exists in some groups of Scrophularia. As early as the beginning of the last century, botanists had carried out a preliminary study on the stigma closure of flowers. They thought that the stigma closure was mainly caused by the reduction of the stigma cell pressure caused by the absorption of water by pollen when it germinated on the stigma; Some hypotheses about the adaptability of stigma closure were put forward. Stigma is the part of flowering plants that directly receives pollen. Its functions include capturing pollen, providing water and nutrients for pollen germination and pollen tube elongation, and promoting pollen tube growth to reach ovules to complete fertilization and so on. In the long-term evolution, the stigmas of plants show a variety of shapes, such as flaky, round, flat trumpet and horseshoe shaped stigmas, and some stigmas are attached with hairy or prickly structures, which are generally conducive to the stigma receiving pollen. At the same time, the stigmas of some plants also show different movement behaviors, such as style curling. The adaptive significance of these diverse stigma behaviors can be summarized as follows: avoiding self-pollination, sincerely reducing the interference of male and female functions, delaying self-pollination, and coping with adverse ecological environment.

Some plants have stigmas (usually two-lobed) that close after being touched by pollinators and then reopen after a certain period of time. Such touch-sensitive stigmas are called touch-sensitive stigmas [1,2]. Plants with touch-sensitive stigmas are widely distributed in the highly core Lamiformes, and specifically include Vitilaceae, Osteophyllaceae, Tongquanaceae, Motherwort and so on. Comparing the closing behavior of the touch-sensitive stigma of different plants, it is found that there is a rich diversity, which is mainly reflected in the three time periods of the closing behavior of the touch-sensitive stigma. That is, the time from when the stigma is touched until it is completely closed [3,4]. The temporary closing time of the same plant is relatively stable, while the variation range between different plants is generally a few seconds to tens of seconds; the reopening time, that is, the stigma is completely closed when it is touched [5,6]. From the beginning to the time of fully reopening, the reopening time of the same plant is relatively stable, and the variation range between different plants is generally a few minutes to tens of minutes; the permanent closure time, that is, after the stigma is pollinated and begins to undergo temporary closure and reopening, The time that the permanent closure and no longer open spontaneously occurs, and the variation range of different plants is generally tens of minutes to several hours [7-9]. Compared with the temporary closure time and re-opening time, the variation range of the permanent closure time of the same plant touch-sensitive stigma is also more likely to be within a few hours. And the time of permanent closure is affected by the amount of pollen and the source of pollen (selfing/outcrossing) [10-12]. The standard of stigma touch sensitivity is not static, but will be affected by various factors. The influencing factors have been found to be: different plant species and different plant populations. The sensitive parts of the touch-sensitive stigma of different plants are also different [13,14]. The sensitivity of the touch sensitive stigma also has certain limits, and excessive or frequent stimulation will reduce or completely lose its sensitivity. At the same time, the sensitivity intensity of stigma is constantly changing throughout the day. Therefore, there may be a positive correlation between the sensitivity of stigma and the light intensity of the sun.

In order to determine the sensitive area of the stigma, many researchers use different instruments to detect the stigma. Generally speaking, stigmas close after pollination, and then reopen within a certain time, and then close again. This phenomenon is commonly found in plant groups with touch sensitive stigmas. Lu (1911) distinguished the closure of stigma into first closure and second closure.

1.2 Touch sensitivity and pollination characteristics of the herb

In this experiment, S. stolonifera, which is widely distributed in central and eastern China, was selected as the research material. When S. stolonifera is low pruned, the creeping cutting glume can produce a fine, precise and well-structured carpet like lawn. It is an excellent cold season lawn grass suitable for golf courses, and can also be used in parks, factories, mines, organs, schools and urban green spaces. It is also a good material for slope protection and soil protection. In previous studies, S. stolonifera had relatively high touch sensitivity in the genus S. spp.; Part of stolon Mazus miquelii is self-incompatible, that is, self-crossing will be fruitful, although it is less than outcrossing, and self-crossing progeny can germinate seedlings and grow and bloom, so it can avoid failure due to self-crossing is not fruitful or does not germinate [15-17]. The DNA of the self-bred progeny was obtained for parental analysis experiments; pollination must be carried out by pollinators to produce fruit, and the types and sizes of pollinators are rich, including: Bee family, Wall bee, Paleia, and Bee. This ensures that there are pollinators of various size gradients, and that the selfed progeny are completely pollinated by the pollinators and not caused by delayed selfing, etc. In the study on the contact sensitivity of stigmas of creeping bentgrass, it was found that compared with large pollinators, the pollination efficiency of small pollinators was lower, and the stigmas contacted required longer time to completely close, and the reopening time was even shorter [18-20]. Artificial mechanical stimulation suggests that, in S. stolonifera, the reaction time for the temporary closure of the stigma may be in response to a mechanical force applied to the stigma: a greater force will result in a shorter time for the temporary closure of the stigma to complete. The stigma behavior of S. stolonifera was significantly different when it was foraged by pollinators of different body sizes. Smaller pollinators, such as stigma, elicited slower responses in stigma closure and shorter duration of temporary closure of stigma [21-23]. This may be due to the limited contact of small pollinators with the stigma and thus little mechanical force applied to the stigma. This argument is supported by the results of artificial mechanical stimulation that small external forces lead to slower response times for stigma closure and shorter duration of temporary closure of the stigma [24-26]. Manual pollination showed that the stigma was permanently closed depending on how much the pollen grains settled. The results show that the factors affecting the reaction time of stigma closing, the duration of stub closing temporarily and the permanent closing of stigma may be different [27]. Previous studies on some plants of Mazus miquelii showed that their stigma tactile sensitivity was significantly different, and it was significantly related to the composition of plant mating system. There are different interaction styles. These results suggest that pollination plays an important role in the adaptive evolution of touch-sensitive behavior in stigmas of this genus. In order to better understand the evolutionary pattern of touch-sensitive stigma and explore the adaptive evolutionary relationship between stigma touch-sensitivity and flower-related traits mediated by pollination, two stolons with different pollination environments and distinct growth differences were selected in this study. The corolla width was used to measure the characteristics of the flower in the population of Mazus miquelii, and the correlation between them was compared and analyzed through the observation of the touch-sensitive behavior of the stigma.

2. Materials and methods

2.1 Research materials and locations

In this study, two wild populations were collected from Donghu Botanical Garden (30°31'N, 114°26'E, 70m) in Hongshan District, Wuhan City, Hubei Province in early March 2022, separated by more than 50m. Plants with good growth were selected for potted cultivation and numbered, with 26 pots in the A population and 14 pots in the B population.

2.2 Flower measurement

The width of the lower lip of the corolla was selected as the representative feature of the flower, and the measurement was performed before recording. The measuring tool was an electronic vernier caliper, and the reading accuracy was 0.1 mm. The inflorescence position was calculated from the sum of shedding and non-shedding flowers under the selected sample.

2.3 Observation of stigma behavior

The closure of the touch sensitive stigma depends on the touch of the pollinator to a large extent. After the stigma is stimulated, it closes rapidly mainly by the movement of the lower lobes. The closure behavior of tactile stigmas has a certain correlation with pollination effectiveness. Only effective pollination can make stigmas close, but it cannot explain the reason why most stigmas close again. In order to compare the differences in stigma touch sensitivity between different populations, this study recorded the closing process of artificial mechanical touching of the stigma. On a sunny afternoon, plants were randomly selected to conduct in vitro experiments on open flowers from the bottom of the inflorescence. Dsam body tone was used for observation and recording (29.96 frames per second), and the center of the lower lobe edge was manually touched with tweezers. In subsequent analysis, the displacement is calculated from the distance from the lower center edge of the lower lobe to the upper center edge of the upper lobe. All stigmas will be observed for pollen falling before measurement, and only stigmas without pollen falling will be selected for measurement.

2.4 Data analysis

All data analyses were performed using SPSS software. The Shapiro-Wilk function was used to test whether the data conformed to a normal distribution, and the Bartlett function was used to test the homogeneity of variances. Shapiro Wilke test is a method to test normality in frequency statistical test. On the one hand, if the p value is less than the selected significance level (the value is usually 0.05), then we should reject the null hypothesis under a higher probability, and the evidence of the data shows that our sample is not from a normal distribution parent. On the other hand, if the p value is larger than the selected significance level, then we have no evidence to reject the null hypothesis, and the data are from a normal distribution. One-way ANOVA was used to compare plant stigma temporal closure time and floral characteristics (corolla width and inflorescence position) when the data fit normal distribution and homogeneity of variance.

3. Result

3.1 Closed or not is significantly correlated with population

Table 1: Proportion of open samples in all samples

Numbering	Total	Open number	Proportion
A17	9	6	66.6%
A22	19	9	47.3%
A24	36	4	11. 1%
A25	12	12	100%
A26	11	0	0%
A27	16	10	62.5%
A6	16	8	50%
B11	27	3	11. 1%
B12	30	1	3.3%
Total	176	53	/

The total number of experimental samples was 176, of which 123 were normally closed and 53 were not closed or incompletely closed (Table 1-3). There is a significant difference in whether it is closed or not in different populations; it also has a significant difference in different pots ($P<0.001$). It is worth noting that all samples in the A26 pot were not completely closed, which will be discussed in a subsequent section.

Table 2: ANOVA univariate analysis of unclosed samples in population A and population B

Sum of square		Degrees of freedom	Mean square	F	Salience
Between groups	4.497	1	4.497	24.044	0
S	32.543	174	0. 187	/	/
Total	37.04	175	/	/	/

Table 3: ANOVA univariate analysis of non-closed samples in each basin group

/	Sum of square	Degrees of freedom	Mean square	F	Salience
Between groups	15.364	8	1.921	14.796	.000
S	21.676	167	0.130		
Total	37.040	175			

3.2 Temporary closure time distribution

According to the statistical results, most of the temporary closing times of the samples in this experiment are between 8.9±5.6 seconds and 2 seconds, and some extreme samples can be as long as 34.1 seconds. Comparing only the normal closed samples (123 samples, 70 samples of population A, and 53 samples of population B), there are differences between different populations and different potted plants ($P<0.001$) (Table 4-6, Figure 1-2).

Table 4: Statistical description of temporary closure time for all samples

/	N	Minimum	Maximum value	Mean	Standard deviation
Time	123	1.77	34.05	8.8576	5.56455
Number of valid cases (in columns)	123	/	/	/	/

Table 5: ANOVA univariate analysis of temporary closure time between populations

Sum of square		Degrees of freedom	Mean square	F	Salience
Between groups	479.439	1	479.439	17.589	.000
S	3298.198	121	27.258	/	/
Total	3777.637	122	/	/	/

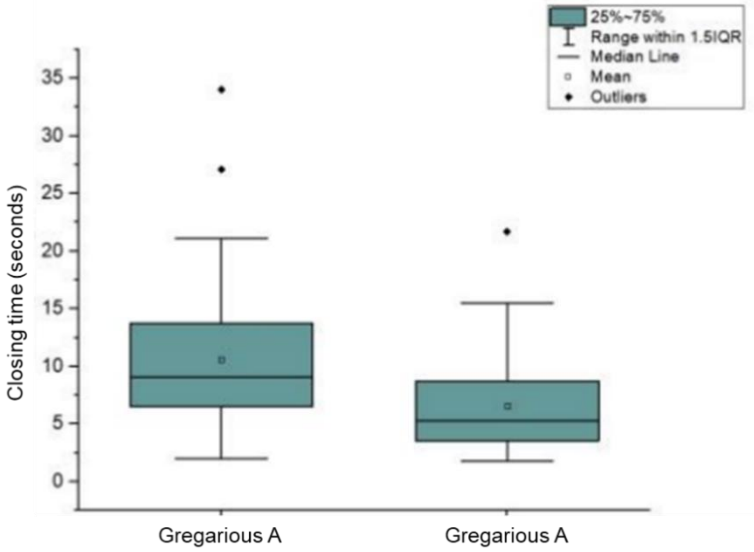

Figure 1: Boxplot of temporary closure time between populations

Table 6: ANOVA univariate analysis of temporary closure time between basin groups

Sum of square		Degrees of freedom	Mean square	F	Salience
Between groups	1105.889	7	157.984	6.800	.000
S	2671.748	115	23.233	/	/
Total	3777.637	122	/	/	/

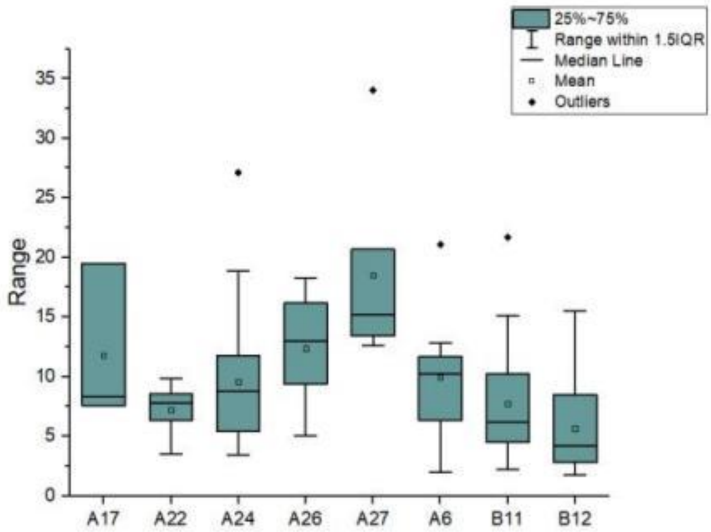

Figure 2: Boxplot of temporary closure time between basin groups

3.3 Temporary closure time was not significantly correlated with flower position and corolla size on different inflorescences

Select pot groups with more than 20 samples, and conduct correlation analysis on temporary closure time, corolla size and inflorescence position within each pot group. In the three pot groups A24, B11, and 12, the temporary closure time was not significantly correlated with inflorescence position and corolla width (Table 7-10).

Table 7: Pearson correlation analysis of temporary closure time with inflorescence position and corolla width

/	/	Inflorescence position	Corolla width
Temporary closing time	Pearson correlation	-0.048	0.039
	Sig. (Two-tailed)	0.602	0.668

Table 8: Pearson correlation analysis of temporary closure time with inflorescence position and corolla width of A24 in the pot group

/	/	Flower location	Corolla	Time
Flower location	Pearson correlation	1	-.447*	-.013
	Sig. (Two-tailed)	/	.010	.945
	Number of cases	32	32	32
Corolla	Pearson correlation	-.447*	1	.165
	Sig. (Two-tailed)	.010	/	.368
	Number of cases	32	32	32
Time	Pearson correlation	-.013	. 165	1
	Pearson correlation	-.013	.165	1
	Sig. (Two-tailed)	.945	.368	/
	Number of cases	32	32	32

Table 9: Pearson correlation analysis of temporary closure time with inflorescence position and corolla width in B11 pot group

/	/	Flower location	Corolla	Time
Flower location	Pearson correlation	1	-.058	.115
	Sig. (Two-tailed)		.767	.553
	Number of cases	29	29	29
Corolla	Pearson correlation	-.058	1	-.054
	Sig. (Two-tailed)	.767		.184
	Number of cases	29	29	29
Time	Pearson correlation	.115	.254	1
	Sig. (Two-tailed)	.553	.184	
	Number of cases	29	29	29

Table 10: Pearson correlation analysis of temporary closure time with inflorescence position and corolla width in B12 pot group

/	/	Flower location	Corolla	Time
Flower location	Pearson correlation	1	-.326	.154
	Sig. (Two-tailed)		.120	.474
	Number of cases	24	24	24
Corolla	Pearson correlation	-.326	1	.414*
	Sig. (Two-tailed)	.120		.044
	Number of cases	24	24	24
Time	Pearson correlation	154	.414*	1
	Sig. (Two-tailed)	.474	.044	
	Number of cases	24	24	24

3.4 Temporary closure time and corolla width decrease from bottom to top on the same inflorescence

The inflorescences with the number of inflorescence flowers greater than or equal to three were selected for observation, and the subsequence lengths that conformed to the strict decreasing trend were counted. In the sample of this experiment, the number of inflorescences with three flowers was 7, and the number of inflorescences with five flowers was 3. In terms of temporary closure time, 5 inflorescences with 3 flowers meet strict decrement, and 2 inflorescences with 5 flowers meet the decrement of subsequence with length 4 (that is, the value of one flower is excluded, and the other four flowers meet strict decrement). This indicated that from the bottom to the top of the inflorescence, the temporary closure time and the width of the corolla showed a decreasing trend (Table 11-12).

Table 11: Regular distribution of temporary closure time in inflorescence from bottom to top

Number of flowers on inflorescence	Number of inflorescences	Longest strictly decreasing subsequence length	The number of inflorescences conforming to strict decrement	Second longest strictly decreasing subsequence length	Meet the second longest strict delivery
3	7	3	5	2	2
5	3	4	2	3	1

Table 12: Regular distribution of corolla width in inflorescence from bottom to top

Number of flowers on inflorescence	Number of inflorescences	Longest strictly decreasing subsequence length	The number of inflorescences conforming to strict decrement	Second longest strictly decreasing subsequence length	Number of inflorescences that conform to the second-longest strictly decreasing
3	7	3	4	2	3
5	3	5	1	4	2

3.5 There are different closing modes

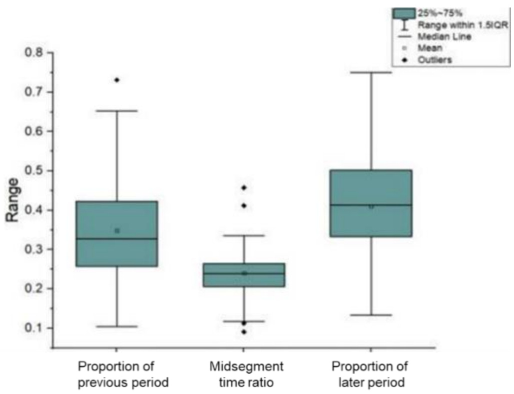

Figure 3: Boxplots of the temporary closing time of each segment after normalization

According to the experimental design, the time used to detect the displacement of the first third, the middle third and the posterior third is normalized according to the total time. It can be found that no matter the total time is long or short, the time used in the front and rear segments is generally significantly longer than that in the middle segment (Figure 3, Figure 4).

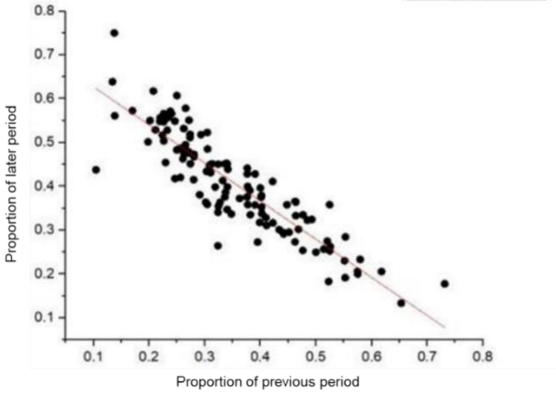

Figure 4: The scatter plot of the proportion of the previous period and the proportion of the latter period, most of the sample points are distributed near the mean, but also obviously spread to both sides

3.6 Correlation of closure pattern with population, corolla, inflorescence position, and temporal closure time

Table 13: Pearson correlations between closure patterns and population, corolla, inflorescence position, and temporary closure time

/	/	Population	Pot number	Flower location	Corolla	Time
Startup speed	Pearson correlation	0. 142	-0. 111	0. 131	0.02	0.003
	Sig. (Two-tailed)	0. 116	0.223	0. 148	0.826	0.974
Mid speed	Pearson correlation	-0.022	0.068	0. 141	-0.026	0. 101
	Sig. (Two-tailed)	0.806	0.454	0. 12	0.774	0.264
Back speed	Pearson correlation	-0. 135	0.081	-0.201	-0.008	-0.051
	Sig. (Two-tailed)	0. 135	0.371	0.026	0.929	0.577

Table 14: Pearson correlation analysis of A24 closure pattern with inflorescence position, corolla width and temporary closure time in the pot group

/	/	Flower location	Corolla	Time
Scale 1	Pearson correlation	-.091	.253	.136
	Sig. (Two-tailed)	.620	.162	.459
	Number of cases	32	32	32
Scale 2	Pearson correlation	.436*	-.541**	-.105
	Sig. (Two-tailed)	.013	.001	.566
	Number of cases	32	32	32
Scale 3	Pearson correlation	-.112	-.014	-.097
	Sig. (Two-tailed)	.543	.937	.597
	Number of cases	32	32	32

The correlation between the closure pattern and the population, corolla, inflorescence position, and temporary closure time was statistically analyzed in the pot group with more than 20 samples; no significant correlation was found (Tables 13-15).

Table 15: Pearson correlation analysis of B11 closure pattern with inflorescence position, corolla width and temporary closure time in the pot group

/	/	Flower location	Corolla	Time
Scale 1	Pearson correlation	.149	.175	.351
	Sig. (Two-tailed)	.439	.363	.062
	Number of cases	29	29	29
Scale 2	Pearson correlation	.144	.052	.103
	Sig. (Two-tailed)	.458	.790	.596
	Number of cases	29	29	29
Scale 3	Pearson correlation	-.223	-.213	-.427*
	Sig. (Two-tailed)	.245	.266	.021
	Number of cases	29	29	29

4. Discuss

4.1 Relationship between temporary closure time and corolla width and inflorescence position

Mazus miquelii is an infinite inflorescence, and the flowers open sequentially from bottom to top. The experimental approach taken in this experiment was to measure all open flowers on the inflorescence at the same time point. In terms of flower characteristics, it can be observed that the temporary closure time of the stigma shows an obvious decreasing trend along the sequence from bottom to top of the inflorescence, that is, the width of the corolla that is newly opened and closer to the top is smaller. At the same time, in the comparison of all sample results, there is no correlation between corolla width and inflorescence position; this does not mean that in the same inflorescence, corolla width is randomly distributed. According to the scheme design of this experiment, the size of the corolla when the buds open, and the continued growth after the corolla opens; which of the two mainly affects the flower characteristics of the samples during observation cannot be determined. At the same time, according to the experimental results, even among different inflorescences in the same pot group, the temporary closure time of the stigma was quite different. On the same inflorescence, the stigma temporary closure time showed an obvious decreasing trend along the bottom-up sequence of the inflorescence.

4.2 Sources of closed mode differences

According to the observed phenomena, the responses of the stigma can be divided into the following categories: (1) Mechanical stimulation of any intensity cannot cause the stigma to close; (2) The stigma does not close completely, but can move normally in the first half (can start normally); (3) The startup will be triggered after a slight touch, and the time used in the acceleration phase and the deceleration phase is equivalent, and the total time is shorter (the touch sensitivity is better); (4) After a slight touch, the startup will be triggered, and the acceleration will be accelerated The time used in the stage and the deceleration stage is similar, but the time used in the three stages is longer, so the total time is longer (weak start and low top speed); (5) After a slight touch, the start will be triggered, and used in the acceleration stage The time is longer, but the other two stages take a short time (weak start but subsequent gradual acceleration)6 triggers a start after a light touch, and only takes longer in the latter part (normal start but weak subsequent acceleration or resistance is encountered inside the lobes). According to previous studies, there is definite experimental evidence that the strength of manual mechanical stimulation will cause a large difference in the closing behavior of stolon vulgaris. In follow-up experiments, it is possible to explore whether there is a link between stimulus intensity and initiation reaction time (the duration of no movement after touch), and the curling process (fast or slow acceleration after initiating movement). The age of flowers and the temperature and humidity of the surrounding environment will also affect the sensitivity of stigma. The stigma closure reaction of plants is generally slow in the case of flowers with long flowering time or in the case of high humidity. It can be seen from the above that there are certain differences in the movement behavior of the touch sensitive stigmas of different groups of plants. Therefore, it is difficult to describe and explain the movement behavior of the touch sensitive stigma with the same fixed pattern.

4.3 Stimulation intensity and stimulation area

In the experiment, the lower lobes were contacted by the tip of the forceps (about 0.5 mm in diameter), and the pressing force did not cause the overall deformation of the lower lobes, but only contacted the mastoid cells on the surface. In previous studies, there were clear images of mastoid cells; more than one hundred mastoids were arranged on the ventral side of the substigma lobes under in vivo audition. Compared to the action-triggering mechanism of the Venus flytrap: when two of the three cilia on the leaf are touched in a short period of time, the leaf will begin to contract. The manual stimulation used in this experiment did not control the exact number of mastoid cells touched, nor the exact force applied.

In this paper, we think that in the future research, we should combine the theory and methods of cell biology and molecular biology to conduct in-depth research. Finally, it is worth mentioning that the movement pattern of flower closure is closely related to its ecological environment. Therefore, the adaptation and evolution of touch sensitive stigmas to their habitats are also worth further exploration.

5. Conclusion

In this experiment, two populations of Rhizoma stolonifera in Wuhan Botanical Garden were used as the experimental objects, which were planted in a laboratory with no pollinators and good light and ventilation. The open stigma was selected for uniform mechanical stimulation, and the closing process of the stigma was recorded. Combined with the characteristics of the flower and the number of flowers on the inflorescence, the law of the temporary closing time of the stigma was summarized, and the movement pattern of the closing process was analyzed. The experimental results show that there are significant differences in the temporary closure time of stigma in different populations; the temporary closure time on the same inflorescence has a decreasing trend in the order from bottom to top; At the same time, there were also different movement patterns, and the differences in movement patterns were not significantly correlated with the population, flower characteristics, and flower positions on the inflorescence. The source of the difference in movement patterns needs to be further studied.

In T. stolonifera, only the lower lobes could feel the mechanical stimulation and respond, so that the stigma was completely closed; however, it could be observed that both the upper and lower lobes were densely covered with papillary cells. If it is assumed that papillary cells on the surface of the lobes are similar to the leaf cilia of Venus flytrap and can convert mechanical stimuli into action potentials, then perhaps the structural basis affecting the conduction of action potentials can be found by comparing the differences in the cellular organization of the upper and lower lobes. In addition, stigma closure is an active movement of plant tissue, and the motor tissue should have characteristics that are different from general plant tissue; between touch-sensitive stigma tissues of different species, as well as Venus flytrap, pitcher plant, sundew, etc. Comparing the movement tissues of plants may reveal similarities and differences in structure and mechanism.

In the results of this study, populations and inflorescences were significantly associated with closing behavior; however, the mechanisms responsible for the differences were unclear. Previous studies have shown that the stigma touch sensitivity of the self-inbred population is gradually lost. In this experiment, it was also found that all the samples in the Yipan group could not be completely closed. If the phenotype of touch sensitivity follows the typical Mendelian inheritance law, can it be speculated that there is an inbred population with stronger touch sensitivity than the general level; or due to other mechanisms, the touch sensitivity of the inbred population must be weakened. These still require further research to determine.

Acknowledgements

The authors would like to show sincere thanks to those techniques who have contributed to this research.

References

[1] Harder, L. D., & Thomson, J. D. (1989). Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. The American Naturalist, 133(3), 323-344.

[2] Heslop-Harrison, J. (1979). Pollen-stigma interaction in grasses: a brief review. New Zealand Journal of Botany, 17(4), 537-546.

[3] Harder, L. D., & Wilson, W. G. (1998). Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. Ecology, 79(8), 2789-2807.

[4] Hülskamp, M., Kopczak, S. D., Horejsi, T. F., Kihl, B. K., & Pruitt, R. E. (1995). Identification of genes required for pollen-stigma recognition in Arabidopsis thaliana. The Plant Journal, 8(5), 703-714.

[5] Roulston, T. A. H., Cane, J. H., & Buchmann, S. L. (2000). What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? Ecological monographs, 70(4), 617-643.

[6] Preuss, D., Lemieux, B., Yen, G., & Davis, R. W. (1993). A conditional sterile mutation eliminates surface components from Arabidopsis pollen and disrupts cell signaling during fertilization. Genes & development, 7(6), 974-985.

[7] McInnis, S. M., Desikan, R., Hancock, J. T., & Hiscock, S. J. (2006). Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytologist, 172(2), 221-228.

[8] Smith-Huerta, N. L., & Vasek, F. C. (1984). Pollen longevity and stigma pre-emption in Clarkia. American Journal of Botany, 71(9), 1183-1191.

[9] Bhattacharya, A., & Mandal, S. (2004). Pollination, pollen germination and stigma receptivity in Moringa oleifera Lamk. Grana, 43(1), 48-56.

[10] Campbell, D. R. (1989). Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. Evolution, 43(2), 318-334.

[11] Cayenne Engel, E., & Irwin, R. E. (2003). Linking pollinator visitation rate and pollen receipt. American Journal of Botany, 90(11), 1612-1618.

[12] Harder, L. D., & Wilson, W. G. (1994). Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. Evolutionary Ecology, 8(5), 542-559.

[13] Thomson, J. D., Draguleasa, M. A., & Tan, M. G. (2015). Flowers with caffeinated nectar receive more pollination. Arthropod-Plant Interactions, 9(1), 1-7.

[14] Ladd, P. G. (1994). Pollen presenters in the flowering plants—form and function. Botanical Journal of the Linnean Society, 115(3), 165-195.

[15] Howell, G. J., Slater, A. T., & Knox, R. B. (1993). Secondary pollen presentation in angiosperms and its biological significance. Australian Journal of Botany, 41(5), 417-438.

[16] Sarkissian, T. S., & Harder, L. D. (2001). Direct and indirect responses to selection on pollen size in Brassica rapa L. Journal of Evolutionary Biology, 14(3), 456-468.

[17] Ge, L. L., Tian, H. Q., & Russell, S. D. (2007). Calcium function and distribution during fertilization in angiosperms. American Journal of Botany, 94(6), 1046-1060.

[18] Richardson, S. C. (2004). Benefits and costs of floral visitors to Chilopsis linearis: pollen deposition and stigma closure. Oikos, 107(2), 363-375.
[19] Galen, C., Shykoff, J. A., & Plowright, R. C. (1986). Consequences of stigma receptivity schedules for sexual selection in flowering plants. The American Naturalist, 127(4), 462-476.
[20] Cheng, H., Wei, J., & Cheng, Z. (2022). Study on sedimentary facies and reservoir characteristics of Paleogene sandstone in Yingmaili block, Tarim basin. Geofluids, 2022.
[21] Blackmore, S., Wortley, A. H., Skvarla, J. J., & Rowley, J. R. (2007). Pollen wall development in flowering plants. New phytologist, 174(3), 483-498.
[22] Han, J., Cheng, H., Shi, Y., Wang, L., Song, Y., Zhnag, W. (2016) Connectivity analysis and application of fracture cave carbonate reservoir in Tazhong. Science Technology and Engineering, 16(5), 147-152.
[23] Zinkl, G. M., Zwiebel, B. I., Grier, D. G., & Preuss, D. (1999). Pollen-stigma adhesion in Arabidopsis: a species-specific interaction mediated by lipophilic molecules in the pollen exine. Development, 126(23), 5431-5440.
[24] Hanlie Cheng, Dong Yang, Changsong Lu, Qiang Qin, David Cadasse, "Intelligent Oil Production Stratified Water Injection Technology", Wireless Communications and Mobile Computing, vol. 2022, Article ID 3954446, 7 pages, 2022.
[25] Cariveau, D., Irwin, R. E., Brody, A. K., Garcia-Mayeya, L. S., & Von Der Ohe, A. (2004). Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. Oikos, 104(1), 15-26.
[26] Hou, Z. K., Cheng, H. L., Sun, S. W., Chen, J., Qi, D. Q., Liu, Z. B. (2019) Crack propagation and hydraulic fracturing in different lithologies. Applied Geophysics, 16(2), 243-251.
[27] Harder, L. D. (1990). Pollen removal by bumble bees and its implications for pollen dispersal. Ecology, 71(3), 1110-1125.