Botanical Studies (2010) 51: 325-336.

Stomatal clustering, a new marker for environmental perception and adaptation in terrestrial plants

Yi GAN, Lei ZHOU, Zhong-Ji SHEN, Zhu-Xia SHEN, Yi-Qiong ZHANG, and Gen-Xuan WANG*

Institute of Argo-Ecology and Eco-Engineering, College of Life Science, Zhejiang University, Hangzhou, 310058, P. R. China

(Received February 12, 2008; Accepted February 26, 2010)

ABSTRACT. "Stomatal clustering," an abnormal stomatal patterning that is formed by two or more stomata in the leaf epidermis, has been reported in more than 60 species of terrestrial plants. According to the characters and distributional pattern, two different types of stomatal clusters were identified, However, calculation of R-values in 16 different plant species showed that the classical method in spatial ecology study could not distinguish between these two types of clusters. Therefore, to classify them, the term "contiguous cluster" and "non-contiguous cluster" were introduced. Their formation and ecological significance were also discussed. In order to study whether stomatal clustering occurs in response to the environment, *Vicia faba* L. were cultivated under different water/salinity levels. Epidermis bioassay was conducted 2 weeks after the treatment. The results showed that drought and salt stresses significantly increased the stomatal density and stomatal index. More importantly, the occurrences of contiguous stomatal clustering also raised along the drought/salt gradients. The result suggests that the stomatal clustering is correlated with environmental signals. It could serve as a new marker for environmental adaptation in terrestrial plants.

Keywords: Environmental stress; One cell spacing rule; Stomatal clustering; Stomatal development; *Vicia faba* L.

INTRODUCTION

Stomata are small pores located on the abaxial and adaxial sides of plant leaves; they are composed of a pair of guard cells and several subsidiary cells. This highly specialized cellular apparatus regulates the plant's carbon dioxide assimilation and water vapor loss through changes in guard cell turgor and stomatal aperture. This is of great importance to the global carbon-water cycle and to the plant's ability to respond to environmental change (Willmer and Fricker, 1996; Hetherington and Woodward, 2003). For the past 50 years, it has been well known that the two-dimensional stomatal distribution during leaf expansion is closely correlated with living environments: plants growing in arid areas tend to have fewer stomata (Croxdale, 2000; Zhang et al., 2003). Elevation of atmospheric carbon dioxide concentration often results in lower stomatal density (Woodward, 1987). At any rate, in most terrestrial plants, planar stomatal distribution is not random (Larkin et al., 1997). There exists a stomata-free region around each stoma, which means at least one intervening epidermal cell is always placed between two neighboring stomata. This is also known as the "one cell spacing rule" which minimizes the overlaps of stomatal gaseous diffusion shells (GDS) and thus ensures the optimal balance between water loss and carbon assimilation (Korn, 1993; Larkin et al., 1997).

However, abnormal stomatal patterning, or "stomatal cluster" as it is known, has been reported successively in certain species of *Begonia*, Crassulaceae, Sonneratiaceae, and Moraceae (Payne, 1970; Metcalfe and Chalk, 1979; Hoover, 1986; Chen, 1996; Tang et al., 2002). This distribution is quite different from the normal stomatal patterns (Payne, 1970). Such stomatal clusters were reported in more than 60 species in Gymnosperm, Dicots, and Monocots (Metcalfe and Chalk, 1979) (see Appendix). The classification and morphological characters of those clusters have received less attention in recent decades. Interestingly, after a review of the literature and a microscopic observation, we actually found two types of stomatal clusters among those plants: type A clusters have 2 (or more) stomata placed in direct contact (without intervening epidermal cells between neighboring guard cells), such as: *Alysicarpus procumbens* Schindl. (Papilionaceae) (Kothari and Shah, 1975; Nilamoni and Parukutty, 1979), Sonneratiaceae. *alba* J. Smith (Chen, 1996), Annonaceae. *hongkongensis* (Sun et al., 1999). While type B clusters are formed by groups of stomata that do not contact with each other (they are separated by the subsidiary cells). Those plants are Crassulaceae. *lineare* (Zheng and Gong, 1999), *Himantandra parvifolia* Bak (Baranova, 1972) and so on (see Appendix 1).

*Corresponding author: E-mail: wanggx@zju.edu.cn; Tel: 86-0571-88206590; Fax: 86-0571-88206590.

These two types of clusters are still not well classified: the term "stomatal cluster" has been used for both types in many studies (Sachs, 1994; Tang et al., 2002; Zhao et al., 2006). Therefore, a clear definition and classification must be redrawn. In the present study, we collected the leaves of 16 plant species which contain normal stomatal patterning and the two types of clusters. Then we used the classical methods which were developed to assess stomatal distribution in ecological studies (Clark and Evans, 1954; Korn, 1993) to evaluate the difference between thesetwo stomatal cluster types.

Interestingly, many plants found to have stomatal clusters were living in arid, salty, or otherwise adverse environments: laterally contiguous stomata were found on the epidermis of *Melilotus suaveolens* Ledeb., which was sampled on the beach at Hangzhou Bay (Figure 1D). Similar clusters were also reported in a typical halophyte *Sonneratia alba* J. Smith (Sonneratiaceae) (Chen, 1996). *Sedum dfredii* Hance, which exhibits stomatal clusters, is a typical CAM plant with strong stress tolerance (Payne, 1970). However, fewer studies have been conducted to address questions like why so many plants bear stomatal

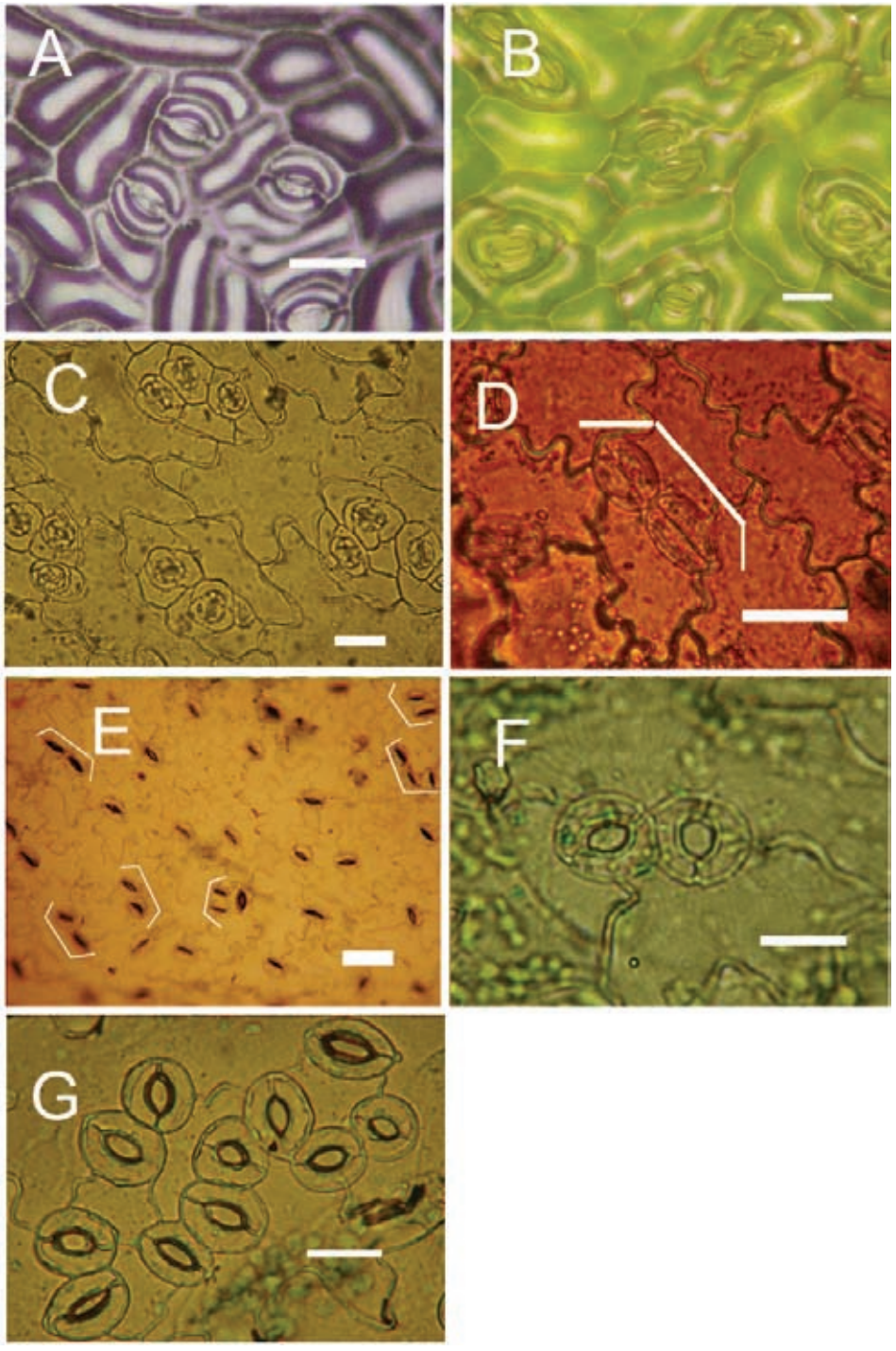

Figure 1. Stomatal clustering in various plant species: (A) Nail polisher imprint show laterally contiguous stomata in *Cinnamomum camphora* (Lauraceae); (B) Non-contiguous stomatal clusters in *Rieger begonia* (Begoniaceae); (C) Non-contiguous stomatal clusters in *Sedum dfredii* Hance (Crassulaceae); (D) Polarly contiguous stomata occasionally seen in *Melilotus suaveolens* Ledeb (*Leguminosae*); (E) Contiguous stomatal clustering in the leaf epidermis of *Vicia faba* Linn. (*Leguminosae*) under water stress; (F) Laterally contiguous stomata occasionally seen in *Arabidopsis thaliana* L. (*Brassicaceae*) Col-0 ecotype; (G) Large contiguous stomatal clusters in the leaf epidermis of *Arabidopsis* mutant line *too many mouths*. Stomatal clusters were marked with white cases. Bars in the pictures represent for 25 μm.

clusters? Whether the occurance of stomatal clusters is a response to the environment or just a coincidence during leaf development in so many terrestrial plants? Moreover, what is the ecological significance of stomatal clustering in the natural environment? In our experiments, we hypothesized that the stomatal clusters could be induced by certain degrees of drought or salt stress and bear a different ecological significance according to the type of clustering. To testify the hypothesis, we chose *Vicia faba* L. and cultivated it in three different soil moisture and salt stress levels. Stomatal density (SD), stomatal index (SI), and the occurrence of stomatal clustering for each treatment were recorded and analyzed.

MATERIALS AND METHODS

Plant material and growth condition

For test of R-value, leaves of 16 different plant species (Table 1) were collected in the Hangzhou Botanical Garden (HZBG) and Zijingang campus (ZJG) at Zhejiang University, during May to July, 2006. *Sesbania grondiflora* (No. 13) was sampled at QuanTang located on the north shore of Hangzhou Bay, where the seawater salinity is 19‰. Wild type (Col-0) and *too many mouths* (*tmm*) mutant lines of *Arabidopsis thaliana* were obtained from Arabidopsis Biological Research Center (ABRC, USA).

In the drought/salt stresses experiment, *Vicia faba* L. seeds were surface sterilized by soaking in 3% hydrogen peroxide for 15 min and then rinsed 5 times with sterile water. Then, they were transferred into 12-cm diameter plastic pots with vermiculite, perlite medium, and fertile soil (BeiLei Ltd., China) mixed at a 1:1:1 proportion. The cultivars were cultivated from seeds in a green house with an 10-h light photoperiod, 18-20°C average daily temperature, and 70% average humidity for 3 weeks before drought and salt treatment.

Test of R-value in the leaves

According to the previous literature, a statistical method is often used to measure and classify stomatal distribution (Clark and Evans, 1954; Korn, 1993). The R-value that refers to the degree of observed distribution departs from random expectation is the key parameter in this method. As described by Clark and Evans, the R-value was calculated using the following formula:

$$\bar{r}A = \frac{\sum r}{N} \quad (1)$$

$$\bar{r}E = \frac{1}{2\sqrt{\rho}} \quad (2)$$

$$R = \frac{\bar{r}A}{\bar{r}E} \quad (3)$$

where ρ is the stomatal density in the measured area r is the distance between the two nearest neighboring stomata, N stands for the number of randomly measured distances between stomata (here is 15-20), $\bar{r}A$ is the mean distance between 2 nearest stomata in an given area. $\bar{r}E$ is the expected distance in an random patterning. When the stomatal distribution is random, the R-value is 1. If the stomatal distribution is clustered, the R-value is near 0, and when distribution is ordered, the *R*-value should be significantly greater than 1 (Croxdale, 2000). In the present study, six replicas of the abaxial surface of leaves were prepared by applying clear nail polish to the surface and

Table 1. 16 different species for leaf R-value test.

No.	Family	Plant	Site
1	Thelypteridaceae	*Macrothelypteris toressiana*	HZBG
2	Pteridaceae	*Pteris multifida*	HZBG
3	Cryptomeria	*Keteleeria oblonga* Cheng	HZBG
4	Cryptomeria	*Metasequoia glyptostostroboides*	ZJG
5	Cycadaceae	*Cycas revoluta* Thunb.	ZJG
6	Aquifoliaceae	*Ilex cornuta*	ZJG
7	Apocynaceae	*Nerium indicum* Mill.	HZBG
8	Salicaceae	*Salix babylonica* Linn.	ZJG
9	Oxalidaceae	*Trifolium pratense* Linn.	ZJG
10	Leguminosae	*Sesbania grondiflora*	ZJG
11	Cruciferae	*Arabidopsis thaliana* (Col-0)	ABRC
12	Nymphaea	*Nymphaea tetragona*	HZBG
13	Lauraceae	*Cinnamonum camphora*	ZJG
14	Leguminosae	*Melilotus suaveolens* Ledeb.	QuanTang
15	Ginkgoaceae	*Ginkgo biloba* L.	ZJG
16	Cruciferae	*Arabidopsis thaliana* (*too many mouths*)	ABRC
17	Crassulaceae	*Sedum dfredii* Hance	ZJG

peeling the film off when dry. The casts were mounted for examination by light microscopy (Nikon E600, 400-fold magnification). 30 pictures were randomly taken. Then, the distance (r) among 15-20 nearest neighboring stomata were measured in each picture with ARCVIEW GIS 3.0 (Environmental Systems Research Institute, Inc. USA).

Drought and salt treatment

To control the soil moisture level, irrigation was ceased 3 weeks after seeding of *V. faba*. A soil moisture meter sensor HH2 (Equipped with WET1 sensor, Delta-T Devices Ltd. UK) was used to monitor the water status. Soil moisture were controlled in three levels, that is 75%-85% field water content (FWC) as control (C), 70-60% FWC as mild drought (M) and 50%-45% FWC as severe drought (S). Water shortage caused by soil evaporation and plant transpiration in each level was precisely compensated each day at a fixed time. The drought treatment lasted for 10-14 days before stomatal patterning analysis. In order to simulate the salty environment and control the soil salinity, 3 weeks after seeding, 0, 0.5%, 1% (W/V) concentration of NaCl solution were added to the cultivar. 10-15 days after salt treatment, the 7th leaves from basal part of the plant were excised, and the epidermis was peeled off and analyzed with the microscope.

Assessment of stomatal patterning

After the drought/salt treatment, ten fully expanded leaves (the 7th leaves from basal part) were randomly chosen, and the abaxial epidermis was peeled off. Then, the epidermis was mounted under the microscope (NIKON E600, 400 fold magnification) and 100 fields of 0.04 square millimeters were randomly selected, and the number of guard cells (N_g) and epidermal cells (N_e) in each field were counted. Stomatal density (SD) per mm^2 and stomatal index (SI) were calculated as follows:

$$SD = 2N_g / 0.04 \quad (4)$$

$$SI = 2N_g/(2N_g+N_e) \quad (5)$$

In addition, the occurrence of stomatal clusters in 100 random selected fields was also counted. The incidence of stomatal clusters under each stressful environment was checked with Pearson Chi-Square Test (χ^2, df=2, p=0.01) in SPSS 16.0 (SPSS Inc. USA).

RESULTS

R-value could not distinguish two types of stomatal clusters

With this method, we tested R-values in 16 species of plants (Table 2) and found most plants with normal stomatal distribution had higher R-values (R>1). In some plants which got contiguous stomatal clusters, R-values were still larger than 1 (No.13-15 in the Table 2). This is because the occurrence of such stomatal clusters was very low in those plants. In contrast, R-values were significantly lower than 1 in other plants exhibiting clusters (No.16, 17in Table 2). From Table 2, it was apparent that the

Table 2. R-values in 16 observed plant species.

No.	Species	R Value	Distribution
1	*Macrothelypteris toressiana*	1.43 ± 0.30	Random
2	*Pteris multifida*	1.37 ± 0.26	Random
3	*Keteleeria oblonga* Cheng	1.72 ± 0.31	Random
4	*Metasequoia glyptostostroboides*	1.16 ± 0.24	Random
5	*Cycas revoluta* Thunb.	1.25 ± 0.25	Random
6	*Ilex cornuta*	1.70 ± 0.30	Random
7	*Nerium indicum* Mill.	1.21 ± 0.27	Random
8	*Salix babylonica* Linn.	1.35 ± 0.23	Random
9	*Trifolium pratense* Linn.	1.39 ± 0.31	Random
10	*Sesbania grondiflora*	1.40 ± 0.23	Random
11	*Arabidopsis thaliana* (Col-0)	1.06 ± 0.18	Random
12	*Nymphaea tetragona*	1.51 ± 0.25	Random
13	*Cinnamonum camphora*	1.42 ± 0.23	Contiguous Cluster
14	*Melilotus suaveolens* Ledeb.	1.35 ± 0.27	Contiguous Cluster
15	*Ginkgo biloba* Linn.	1.36 ± 0.32	Contiguous Cluster
16	*Arabidopsis thaliana* (*tmm* mutant)	0.57 ± 0.08	Contiguous Cluster
17	*Sedum dfredii* Hance	0.56 ± 0.16	Non-contiguous Cluster

The majority of plants with a random stomatal distribution often have an R-value larger than one. In plants that have stomatal clusters, the R-value varies according to the type of clusters. In plants exhibiting contiguous stomatal clusters, the R-value is larger than one (except for *tmm* mutant), but in plants with non-contiguous stomatal clusters, the R value is smaller than 1.

R-values could not distinguish between the two types of stomatal clusters. Thus, we introduced two terms: "contiguous cluster" and "non-contiguous cluster" to distinguish between them. In most cases, the incidence of contiguous clusters is often very low across the leaf blade. Therefore, the R-values are often larger than 1 in those plants (13, 14, 15 in Table 2). Non-contiguous clusters have most of stomata arranged in groups at the whole leaf level (Figure 2). Thus, the R-values are often smaller than 1, but none of those clustered stomata "touch" each other.

Drought and salt stress induces stomatal clustering in the leaf epidermis of *Vicia faba* L.

Drought and salt stress significantly influenced the stomatal patterning (Table 3). Compared with control, the stomatal density (SD) increased 30% and 21% in mild and severe drought stresses, respectively. Meanwhile, the stomatal index (SI) also increased 10.7% and 14.3% ($p<0.05$, n=100), which suggests more stomata were produced in a given area under drought stress as opposed to shrinkage of epidermal cells. In the salinity experiment, similar results were also observed. SD increased 15% and 36% following application of 0.5% and 1% concentration of NaCl solution ($p<0.01$, n=100), Meanwhile, SI increased 9.4% and 15.2% along the salinity gradient, respectively.

More importantly, the incidence of contiguous stomatal clustering in the leaf epidermis also increased along the water ($\chi^2 = 18.667$, df=2, $p<0.01$) and salinity gradient ($\chi^2=10.988$, df=2, $p<0.01$) (Table 3). In the drought experiment, only two clusters were found in the observed 469 stomata of well-watered plants while 8 (in 611 stomata observed) and 22 (in 568 stomata observed) stomatal clusters were found under mild and severe drought stresses, respectively. The incidence of stomatal clustering also increased along the salt gradient. 2, 6 and 14 pairs of stomatal clusters were found in 100 randomly selected fields of the control, 0.5% and 1% salt treatments, respectively. Interestingly, most stomatal clusters observed were composed of two stomata placed side by side or end to end. In all fields of view analyzed, only three bigger clusters (more than three stomata) were found. Such stomatal clusters are very similar to those of the *Arabidopsis thaliana* mutant line *four lips* of which have impaired symmetric divisions of the guard mother cell (GMC) (Yang and Sack, 1995). Such an abnormality has also occasionally been seen in *Arabidopsis thaliana* Col-0 ecotype (Figure 1F).

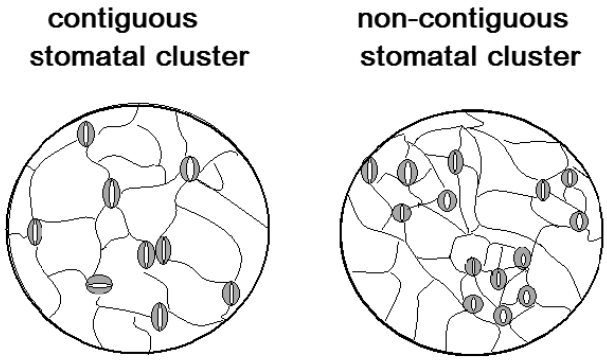

Figure 2. Two different types of stomatal clusters: stomata in contiguous clusters are in direct contact with their neighbors. In non-contiguous clusters, stomata are placed in groups but do not touch each other. Contiguous clusters are occasionally seen in leaves while most stomata are grouped in non-contiguous clusters.

DISCUSSION

The origin of stomatal clustering is environmentally responsive

In model species *Arabidopsis thaliana* L., initiation of guard cells involves formation of the meristemoid mother cell (MMC), which will experience three rounds of asymmetric divisions and form a meristemoid (M) with several larger sister cells. Then, the meristemoid turns into guard mother cells (GMC) and finally become a pair of guard cells (GC) through a symmetric division (Sachs, 1994; Yang and Sack, 1995; Geisler et al., 2000). Interestingly, each time asymmetric divisions are launched, a newly formed meristemoid is usually away from the existing stomata or precursor cells (Figure 3). Such a unique patterning law backs up the "one cell spacing rule."

Table 3. Stomatal patterning in the leaves of *Vicia faba* L. under different levels of drought and salt stresses.

Treatment		SD	SI	Incidence of clustering	Pearson chi-square test
Drought	Well watered	117.25 ± 7.08c	0.28 ± 0.012b	2%	χ^2=18.667
	Mild drought	152.75 ± 8.84a	0.31 ± 0.014a	8%	df = 2
	Severe drought	142 ± 5.93b	0.32 ± 0.012a	20%	p<0.01
Salt	0% Salinity	100 ± 6.41c	0.255 ± 0.015c	2%	χ^2=10.988
	0.5% Salinity	123.75 ± 7.05b	0.306 ± 0.014b	6%	df = 2
	1% Salinity	151.25 ± 8.44a	0.319 ± 0.014a	14%	p<0.01

SD and SI in each treatment were compared with one-way ANOVA (p=0.05). The incidence of clustering along the drought and salinity gradient were checked with Pearson Chi-Square test in SPSS 16.0. (df =2, p=0.01). Data labeled with different letters were statistical different.

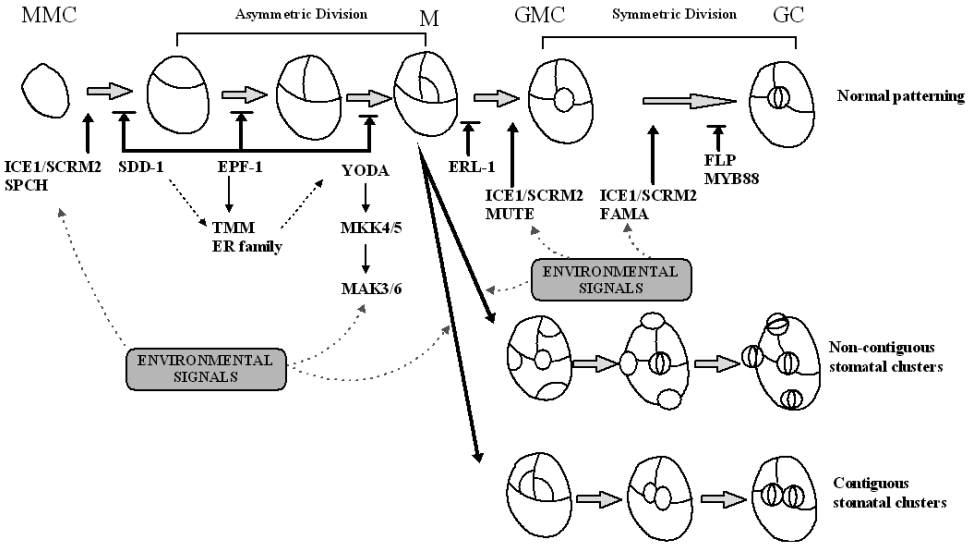

Figure 3. Several genes and transcriptional factors regulate the stomatal lineage in *Arabidopsis thaliana.* Many of them, such as YODA, MKK4/5, MAK3/6 and ICE/SCRM2, are correlated with multiple biotic/abiotic stress responses. Mutation of those genes and transcriptional factors causes excessive formation of meristemoid adjacent to the existing stoma and lead to contiguous stomatal clustering. On the other hand, the formation of non-contiguous stomatal clusters may initiate from the generation of new satellite meristemoid around the existing stoma. This pattern of cell differentiation may be modulated by environmental signals, also.

Over the last 15 years, it has been known that a series of genes and transcription factors regulate the fate of protodermal cells during stomatal differentiation (Figure 3) (Yang and Sack, 1995; Bergmann, 2006; Nadeau, 2009). The TMM, SDD-1, and ERECTA family are required to inhibit the excessive cell divisions between the meristemoid (Geisler et al., 2000; Groll et al., 2002; Shpak et al., 2005). YODA, a Mitogen-activated protein kinase kinase kinase gene, acts as a molecular switch regulating cell identities in the epidermises (Bergmann et al., 2004). MKK4/5 and MPK3/6 act downstream of YODA and negatively regulate the initiation of the meristemoid during asymmetrical division (Wang et al., 2007). Moreover, EPF1 encodes a small secretory peptide that secretes into the protodermal cells and inhibits the formation of a satellite meristemoid (SM) near the precursor cells (Hara et al., 2007). At last, FOUR LIPS (FLP) and its homolog MYB88 control the symmetrical division at the formation of the guard cells (Lai et al., 2005). These genes and proteins negatively control the cell differentiation and eliminate excessive divisions near meristemoids and persecutor cells (Nadeau, 2009). Mutation of them would bring contiguous stomatal clusters of different size and frequency. Recently, three transcriptional factors—SPCH, MUTE and FAMA—were also identified to positively regulate the initiation of asymmetrical and symmetrical divisions in the stomatal lineages (Ohashi-Ito and Bergmann, 2006; MacAlister et al., 2007; Pillitteri et al., 2007). At the same time, ICE1 and SCRM2 are required for the sequential actions of SPCH, MUTE, and FAMA (Kanaoka et al., 2008).

However, we have little information on whether these genes or transcriptional factors are regulated by multiple biotic/abiotic factors or whether the origin of stomatal clusters is affected by long-term climate change (Gray and Hetherington, 2004). It was reported that the density of epidermal cells and stomata in *Scrophularia yoshimurae* increased significantly in the less ventilated growth container (Chen et al., 2006). Serna & Fenoll also reported contiguous stomatal clusters were induced in the c24 ecotype of *Arabidopsis thaliana* growing in a sealed chamber with little gas exchange (Serna and Fenoll, 1997). These results suggest that a gaseous signal (like ethylene or a decrease in atmospheric CO_2 concentration) may affect stomatal patterning. Moreover, the ERECTA gene, which co-regulates stomatal patterning with ERL1 and ERL2 (Shpak et al., 2005), has been found to modify transpiration efficiency under stressful environments (Masle et al., 2005). The environmentally responsive mitogen-activated protein kinases: MPK3/6 and MKK4/5, downstream components of YODA, were shown to regulate stomatal patterning in *Arabidopsis*. Intriguingly, MAPK and MAPKK also play central roles in plant abiotic and biotic stress signaling networks (Asai et al., 2002; Droillard, 2002; Pedley and Martin, 2005; Wang et al., 2007). This finding established a complete MAPK signaling cascade as a key regulator of stomatal development and patterning (Wang et al., 2007). Recently, SCRM2 were found to encode a bHLH leucine zipper protein ICE1 and contribute to freezing tolerance (Kanaoka et al., 2008). Those genes and proteins may serve as crucial candidates for integration of environmental signals into stomata lineages (Figure 3). In the present study, our results also show that osmotic stress caused by drought and salinity impaired normal stomatal patterning and produced more contiguous stomatal clusters in the leaf epidermis. Notably, the presence of such

contiguous clusters in *V. faba* is quite similar to the *Arabidopsis thaliana* mutant line *four lips*, which suggest the symmetric divisions of guard mother cells during stomatal differentiation are probably impaired by osmotic stresses (Figure 3). Components like EPF-1, FLP, and the MAP kinase family may be involved in this process. However, despite the contiguous clusters discussed above, little is known about the origin of non-contiguous stomatal clustering in plants in their natural habitats (Appendix 1). We assume that the appearance of non-contiguous clustering is formed by the generation of a new satellite meristemoid around the primary stoma (Figure 3). They are all arising from one original MMC. Some position-based signals like EPF-1 may contribute to the spatial patterning of the satellite meristemoid and the "one cell spacing rule."

Stomatal clustering is a new marker of environmental perception and adaptation in terrestrial plants

Since adherence to the "one cell spacing rule" could reduce the overlap of stomatal gaseous diffusion shells (GDS) and ensure the optimal balance between carbon assimilation and water loss in plants (Larkin et al., 1997), why do so many plants have stomatal clusters? Did stomatal clusters bear any ecological or evolutional advantage for the adaptation of terrestrial plants under natural selection? A few scientists have proposed that stomatal clusters have positive effects on plants. Hoover studied the ecological response of non-contiguous stomatal clusters in two Mexican *Begonia* species (*B. beracleifolia* & *B. nelumbiifolia*) and found stomatal clusters may assist in water conservation: populations growing on the rocks near waterfalls have larger clusters than those growing on well-watered soil substrates (Hoover, 1986). Tang and his colleges reported a positive relationship between cluster size and multiple epidermis, which is a typical drought adaptation trait in *Begonia peltatifolia* (Tang et al., 2002). Moreover, in *Arabidopsis*, exposed to high light intensities, dark adapted *sdd-1* mutants which have contiguous clusters had 30% higher CO_2 assimilation rates than that of the wild type (Schlüter et al., 2003). Additionally, a negative correlation between the number of contiguous stomatal clusters and soil moisture level has been established in *Cinnamomum camphora* (Lauraceae) (Zhao et al., 2006). Our unpublished experiments on the *too many mouths* mutant of *Arabidopsis thaliana* showed that normal stomatal regulation was impaired by guard cell extrusion in higher number of contiguous stomatal clusters.

In light of the evidence discussed above, it is tempting to speculate that: since most normal stomata are kept in groups, in non-contiguous stomatal clusters, overlaps of GDS could reduce the total area of evaporation shells, and water loss would be kept to a minimum (Figure 4). It may be a long-term adaptation to the ever-challenging environment by keeping to the "one cell spacing rule." On the other hand, based on the cellular hydraulic interaction theory (Mott et al., 1997; Mott et al., 1999), the normal regulation

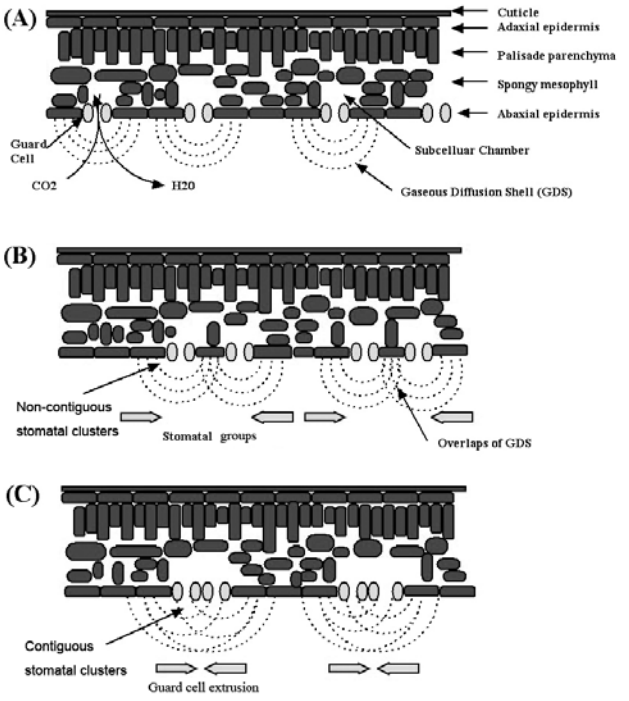

Figure 4. (A) The "one cell spacing rule" reduces the overlaps of Gaseous Diffusion Shells (GDS) in each stoma and balances the carbon assimilation and leaf transpiration; (B) Overlaps of GDS in non-contiguous stomatal clusters may reduce leaf transpiration and keep water loss at a lower level; (C) Direct contact of guard cells in contiguous stomatal clusters may cause cellular extrusion and impair normal stomatal function.

of stomata would probably be impaired due to guard cell extrusion in large contiguous stomatal clusters. Therefore, plants that have larger or a larger number of stomatal clusters (like *too many mouths* mutant of *Arabidopsis*) would be selected in a fluctuating environment. That may explain why contiguous clusters are so rare in most terrestrial plants. In addition, some abnormal environmental responsive gene expression (Like ERECTA, MKK4/5-MPK3/6) may be involved in this process. Contiguous stomatal clusters are more like "scars" on the "faces" of plant leaves instead of an evolutional advance.

In conclusion, stomatal clusters are abnormal patterning in the leaves of many terrestrial plants. Evidence in morphology, gene regulation, and cellular signaling have demonstrated the delicate links between stomatal clusters and environmental factors. Nevertheless, the detailed mechanism still needs further exploration. Stomatal clusters, tiny marks on the leaves, leave us new clues to uncover the truth about the adaptation of terrestrial plants to the ever-challenging environment, ecologically and evolutionally.

Acknowledgement. We sincerely thank the anonymous reviewer for the insightful opinions and improvement of the language. This study is supported by Natural Science Fund of China (project 30871470, 30730020) and Ministry of Science and Technology of China (863 program, 2006AA100202).

LITERATURE CITED

Asai, T., G. Tena, and J. Plotnikova. 2002. MAP kinase signalling cascade in Arabidopsis innate immunity. Nature **415:** 977-983.

Baranova, M. 1972. Systematic anatomy of the leaf epidermis in the magnoliaceae and some related families. Taxon **21:** 447-469.

Bergmann, D.C. 2006. Stomatal development: from neighborly to global communication. Current Opinion Plant Biol. **9:** 478-483.

Bergmann, D.C., W. Lukowitz, and C.R. Somerville. 2004. Stomatal development and pattern controlled by a MAPKK kinase. Science **304:** 1494-1497.

Chen, U.C., C.N. Hsia, D.C. Agrawal, and H.S. Tsay. 2006. Influence of ventilation closures on plant growth parameters, acclimation and anatomy of leaf surface in *Scrophularia yoshimurae* Yamazaki - a medicinal plant native to Taiwan. Bot. Stud. **47:** 259-266.

Chen, Z.L. 1996. The morphology and anatomy of *Sonneratia* Linn in China. J. Tropical Subtropical Bot. **4:** 18-24.

Clark, P.J. and F.C. Evans. 1954. Distance to nearest neighbor as a meature of spatial relationship in populations. Ecology **35:** 445-453.

Croxdale, J.L. 2000. Stomatal patterning in angiosperms. Amer. J. Bot. **87:** 1069-1080.

Droillard, M. 2002. Different protein kinase families are activated by osmotic stresses in Arabidopsis thaliana cell suspensions. Involvement of the MAP kinases AtMPK3 and AtMPK6. FEBS Lett. **527:** 43-50.

Geisler, M., J. Nadeau, and F.D. Sack. 2000. Oriented asymmetric divisions that generate the stomatal spacing pattern in Arabidopsis are disrupted by the too many mouths Mutation. Plant Cell **12:** 2075-2086.

Gray, J.E. and A.M. Hetherington. 2004. Plant development: YODA the stomatal switch. Current Biol. **14:** 488-490.

Groll, U.V., D. Berger, and T. Altmann. 2002. The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during Arabidopsis stomatal development. Plant Cell **14:** 1527-1539.

Hara, K., R. Kajita, K.U. Torii, D.C. Bergmann, and T. Kakimoto. 2007. The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. Genes & Dev. **21:** 1720-1725.

Hetherington, A.M. and F.I. Woodward. 2003. The role of stomata in sensing and driving environmental change. Nature **424:** 901-908.

Hoover, W.S. 1986. Stomata and stomatal clusters in *Begonia*: ecological response in two mexican species. Biotropica **18:** 16-21.

Kanaoka, M.M., L.J. Pillitteri, H. Fujii, Y. Yoshida, N. L. Bogenschutz, J. Takabayashi, J.-K. Zhu, and K.U. Toriia. 2008. SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to arabidopsis stomatal differentiation. Plant Cell **20:** 1775-1785.

Korn, R.W. 1993. Evidence in dicots for stomatal patterning by inhibition. International J. Plant Sci. **154:** 367-377.

Kothari, M.J. and G.L. Shah. 1975. Epidermal Structure and Ontogeny of Stomata in the *Papilionaceae* (Tribe Hedysareae). Bot. Gazette **136:** 372-379.

Lai, L.B., J.A. Nadeau, J. Lucas, E.-K. Lee, T. Nakagawa, L. Zhao, M. Geisler, and F.D. Sack. 2005. The Arabidopsis R2R3 MYB proteins FOUR LIPS and MYB88 restrict divisions late in the stomatal cell lineage. Plant Cell **17:** 2754-2767.

Larkin, J.C., M.D. Marks, J. Nadeau, and F. Sack. 1997. Epidermal cell fate and patterning in leaves. Plant Cell **9:** 1109-1120.

MacAlister, C.A., K. Ohashi-Ito, and D.C. Bergmann. 2007. Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. Nature **445:** 537-540.

Masle, J., S.R. Gilmore, and G.D. Farquhar. 2005. The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. Nature **436:** 866-870.

Metcalfe, C.R. and L. Chalk. 1979. Anatomy of the dicotyledons. Second ed. Oxford: Clarendon Press.

Mott, K.A., F. Denne, and J. Powell. 1997. Interactions among stomata in response to perturbations humidity. Plant Cell Environ. **20:** 1098-1107.

Mott, K.A., J.C. Shope, and T.N. Buckley. 1999. Effects of humidity on light-induced stomatal opening:evidence for hydraulic coupling among stomata. J. Exp. Bot. **50:** 1207-1213.

Nadeau, J.A. 2009. Stomatal development: new signals and fate determinants. Current Opinion in Plant Biol. **12:** 29-35.

Nilamoni, B. and B. Parukutty. 1979. Contiguous stomata in *Desmodium* Desv. (Papilionaceae). Current Sci. (India) **48:** 27-28.

Ohashi-Ito, K. and D.C. Bergmann. 2006. Arabidopsis FAMA controls the final proliferation/differentiation switch during stomatal development. Plant Cell **18:** 2493-2505.

Payne, W.W. 1970. Helicocytic and allelocytic stomata: unrecognized patterns in the dicotyledonae. Amer. J. Bot. **57:** 140-147.

Pedley, K.F. and G.B. Martin. 2005. Role of mitogen-activated protein kinases in plant immunity. Curr. Opin. Plant Biol. **8:** 541-547.

Pillitteri, L.J., D.B. Sloan, N.L. Bogenschutz, and K.U. Torii. 2007. Termination of asymmetric cell division and differentiation of stomata. Nature **445:** 501-505.

Sachs, T. 1994. Both cell lineages and cell interactions contribute to stomatal patterning. Int. J. Plant Sci. **155:** 245-247.

Schlüter, U., M. Muschak, D. Berger, and T. Altmann. 2003. Photosynthetic performance of an Arabidopsis mutant with elevated stomatal density (*sdd1-1*) under different light regimes. J. Exp. Bot. **54:** 867-874.

Serna, L. and C. Fenoll. 1997. Tracing the ontogeny of stomatal clusters in *Arabidopsis* with molecular markers. Plant J. **12:** 747-755.

Shpak, E.D., J.M. McAbee, L.J. Pillitteri, and K.U. Torii. 2005. Stomatal patterning and differentiation by synergistic interactions of receptor kinases. Science **309:** 290-293.

Sun, T., Y. Hu, and K. Lang. 1999. A Study on Micromorphological Characters of Leaf Epidermis of Neottianthe in China. Acta Bot. Yunn. **21:** 57-62.

Tang, M., Y.X. Hu, J.X. Lin, and X.B. Jin. 2002. Developmental mechanism and distribution pattern of stomatal clusters in *Begonia peltatifolia*. Acta Bot. Sin. **44:** 384-390.

Wang, H., N. Ngwenyama, Y. Liu, J.C. Walker, and S. Zhang. 2007. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. Plant Cell **19:** 63-73.

Willmer, C. and M. Fricker. 1996. Stomata. Second ed. London: Chapman & Hall.

Woodward, F.I. 1987. Stomatal numbers are sensitive to increases in CO_2 from preindustrial levels. Nature **327:** 617-618.

Yang, M. and F.D. Sack. 1995. The too many mouths and four lips Mutations Affect Stomatal Pnoduction in *Arabidopsis*. Plant Cell **7:** 2227-2239.

Zhang, X.Y., H.M. Yang, and G.X. Wang. 2003. Stomatal density and distribution of spring wheat leaves under different planting densities and soil moisture levels. Acta Phytoecol. Sin. **27:** 133-136.

Zhao, X.Z., Y.S. Yang, and Z.X. Shen. 2006. Stomatal clustering in *Cinnamomum camphora*. South Africa J. Bot. **72:** 565-569.

Zheng, Y. and J. Gong. 1999. A leaf epidermis study on tweleve species of *Sedum* in Anhui. Bull. Bot. Res. **19:** 292-297.

Appendix 1. Stomatal clustering in terrestrial plants.

Family	Genera	Species	Stomatal cluster type	Incidence of clustering	References*
Gymnosperm					
Ginkgoaceae	*Ginkgo biloba*	*G. biloba* L.	2 or 3 contiguous stomata	Low	Chen and Li, 2004
Zamiaceae	*Zamia*	*Z. furfuracea*	2 or 3 contiguous stomata	Low	Su et al., 2003
Bennettitales	*Tyrmia*	*T. susongensis* sp. nov.	Contiguous stomata	Occasionally seen	Cao, 1998
	Prynada	*Pterophyllum* sp.	Laterally contiguous stomata		
Casuarinaceae	*Casuarina*	*C. equisetifolia*	2 contiguous stomata	Low	Torrey and Berg, 1988
Ephedraceae	*Ephedra*	*E. foliata* Boiss	2 contiguous stomata placed vertically (end to side)	Occasionally seen	Pant and Mehra, 1964
Podocarpaceae	*Dacrydium* *Podocarpus*	*D. balansae* *D. lycopodioides* *P. madagascariensis* *P. woltzii*	Polarly contiguous stomata	Low	Stockey et al., 1998; Stockey and Dacrydium, 1992
Angiosperm (Dicotyledon)					
Apocynaceae	*Aganosma*	*A. dichotoma* K. Schum	Several stomata placed together forming an non-contiguous group	High	Patel et al., 1972
Annonaceae	*Annona* *Artabotrys*	*A. muricata* L. *A. hongkongensis* *Desmos chinensis* *Uvaria macrophylla*	Contiguous stomatal placed end to end	Low	Sun et al., 2001
Asteraceae	*Emilia*	*E. coccinea* *E. sonchifolia*	Contiguous stomata	Low	Ndukwu and Agbagwa, 2006
Amaryllidaceae	*Agapanthus* *Allium* *Amaryllis* *Nerine* *Crinum* *Cyrtanthus* *Cooperia* *Pancratium* *Narcissus*	*A. umbellatus* (L) *A. cepa* (L) *A. tuberosum* (L) *A. belladonna* (U, L) *A. vittata* (U, L) *N. curvij'olia* (L) *C. zeylanicum* (U, L) *C. asiaticum* (L) *C. mackenii* (L) *C. pedunculata* (U, L) *P. verecundum* (L) *N. tazetta* (U)	Laterally or polarly contiguous stomata		Awasthi et al., 1984

Appendix 1. (Continued)

Family	Genera	Species	Stomatal cluster type	Incidence of clustering	References*
Begoniaceae	*Begonia*	*B. cavaleriei* L. *B. heracleifolia* *B. nelumbiifolia* *B. erythrophylla* *B. metallica* *B. semperflorens* *B. aridicaulis* *B. peltatifolia*	4-6 stomata forming a non-contiguous group	High	Dehnel, 1961; Payne, 1970 Hoover, 1986; Tang et al., 2002
Bombacaceae	*Kostermansia*	*K. malayana*	Stomata arranged in groups Juxtaposed contiguous stomata occasionally seen	High	Metcalfe and Chalk, 1979
Celatraceae	*Elaeodendron*	*E. glaucum*	Laterally contiguous stomata	High	Pant and Kidwai, 1966
Crassulaceae	*Sedum*	*S. lineare* *S. aizoon* *S. polytrichoiides* *S. kiangnanense* *S. emargi nat um* *S. alboroseum* *S. dfredii* Hance	2-6 Stomata forming a non-contiguous group	High	Zheng and Gong, 1999; Payne, 1970
Cruciferae	*Brassica* *Lepidium* *Raphanus*	*B. oleracea* *L. virginicum* *R. salivus*	Stomata arranged in non-contiguous groups	High	Payne, 1970
Gesneriaceae	*Gesneria*	*sintenisii* Urb.	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Himantandraceae	*Himantandra*	*H. parvifolia* Bak.	4-6 stomata clustered around the bases of peltate scales	High	Baranova, 1972
Ixonanthaceae	*Irvingia* *Klainedoxa*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Lauraceae	*Cinnamomum*	*C. camphora*		Low	Zhao et al., 2006
Leguminosae	*Melilotus*	*M. albus* Desr.	Polarly or laterally contiguous stomata	Low	First report
Menispermaceae	*Eleutharrhena*	*E. macrocarpa* Forman	30-68 stomata arranged an island, some of them are contiguous	High	Hong et al., 2001
Melastomataceae	*Calycogonium* *Leandra* *Ossaea*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Moraceae	*Ficus*	Some species	Stomata arranged in non-contiguous groups	High	Payne, 1970
Nyssaceae	*Camptotheca*	*C. acuminata* Dence	2 Contiguous stomata near small veins (laterally, polarly or diagonally)	Low	Xi et al., 1997
Nymphaeaceae	*Nelumbo* Adans.	*N. nucifera* Gaertn	2 contiguous stomata	Low	Gupta et al., 1968
Ochnaceae	*Godaya* *Sauvagesia*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Primulaceae	*Primulal*	*P. merrilliana* *P. cicularüifolia*	Laterally contiguous stomata	Occasionally seen	Chen et al., 2004
Papilionaceae	*Aeschynomene* *Alysicarpus* *Arachis*	*Euchresta* *Aeschynomene indica* *Alysicarpus -procumbens* *Alysicarpus -vaginalis* *Arachis hypogaea* *Desmodium* Desv.	2-4 laterally or polarly contiguous stomata	Low	Kothari and Shah, 1975; Nilamoni and Parukutty, 1979

Appendix 1. (Continued)

Family	Genera	Species	Stomatal cluster type	Incidence of clustering	References*
Proteaceae	*Banksia* *Dryandra* *Lambertia*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Rubiaceae	*Pagamea*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Solanaceae	*Capsicum* *Solanum*	*C. annuum* L. *S. melongena* L.	Juxtaposed, superposed, and obliquely placed contiguous stomata	Low	Patel and Shah, 1971
Sonneratiaceae	*Sonneratia*	*S. alba* J. Smith	Contiguous stomata	Occasionally seen	Chen, 1996
Saxifragaceae	*Saxifrage*, *Chrysosplenium*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Simaroubaceae	*Castela* *Soulamea*	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Theaceae	*Cleyera* Thunb.	Some species	Stomata arranged in non-contiguous groups	High	Metcalfe and Chalk, 1979
Verbenaceae	*Stachytarpheta*	Some species	Stomata arranged in non-contiguous groups	High	Payne, 1970
Angiosperm Monocotyledon					
Araceae	*Alocasia* *Arisaema* *Zamioculcas*	Some species	Polarly contiguous stomata	Low	Pant and Kidwai, 1966
Liliaceae	*Asparagus*	*A. gonoclados* Baker *A. plumosus* Baker *A. racemosus* var. *javanicus* Baker *A. sprengeri* Regel.	Polarly or laterally contiguous stomata	Low	Gopal and Shah, 1970
Orchidaceae	*Neottianthe*	*N. angustifolia* *N. calcicola* *N. camptoceras* *N. compacta* *N. cucullata* *N. gymnadenioides* *N. luteola* *N. monophylla* *N. oblanga* *N. ovata* *N. pseudodiphylax* *N. securrdiflora*	Polarly contiguous stomata	High	Sun et al., 1999

***References for Appendix 1.**

Awasthi, D.K., V. Kumar, and R. Rawat. 1984. Stomatal studies in *Amaryllidaceae* with sepcial reference to stomatal abnormalities. Proc. Indian Acad. Sci (Plant Sci.) **93:** 629-633.

Baranova, M. 1972. Systematic anatomy of the leaf epidermis in the *magnoliaceae* and some related families. Taxon **21:** 447-469.

Cao, Z.Y. 1998. A study on the cuticles of some *bennettital eans* from the lower part of Xiangshan group in Jiangsu and Anhui Provinces. Acta Palaeon Tologica Sin. **37:** 283-294.

Chen, L.Q. and C.S. Li. 2004. The epidermal characters and stomatal development of *Ginkgo biloba*. Bull. Bot. Res. **24:** 417-422.

Chen, M.L., X.P. Zhang, and X.H. Li. 2004. Study on micromorphological features of *Primula merrilliana* and *P. cicutariifolia*. J. Plant Res. Environ. **13:** 18-24

Chen, Z.l. 1996. The morphology and anatomy of *sonneratia* linn in China. J. Trop. Subtropical Bot. **4:** 18-24.

Dehnel, G.S. 1961. Abnormal stomatal development in foliage leaves of *Begonia aridicaulis*. Amer. J. Bot. **48:** 129-133

Gopal, B.V. and G.L. Shah. 1970. Observations on Normal and Abnormal Stomatal Features in Four Species of *Asparagus* L. Amer. J. Bot. **57:** 665-669.

Gupta, S.C., G.S. Paliwal, and R. Ahuja. 1968. The stomata of *Nelumbo nucifera:* formation, distribution and degeneration. Amer. J. Bot. **55:** 295-301.

Hong, Y.P., K.Y. Pan, and Z.D. Chen. 2001. Characters of leaf epidermis and their systematic significance in *Menispermaceae*. Acta Bot. Sin. **43:** 615-623.

Hoover, W.S. 1986. Stomata and stomatal clusters in *begonia*: ecological response in two mexican species. Biotropica **18:** 16-21.
Kothari, M.J. and G.L. Shah. 1975. Epidermal structure and ontogeny of stomata in the Papilionaceae (Tribe Hedysareae). Bot. Gazette **136:** 372-379.
Metcalfe, C.R. and L. Chalk. 1979. Anatomy of the dicotyledons. Second ed. Oxford: Clarendon Press.
Ndukwu, B.C. and I.O. Agbagwa. 2006. The value of leaf micromorphological in the taxonomic delimitation of *Emilia cass*. (Asteraceae) species Global. J. Pure Appl. Sci. **1:** 183-187.
Nilamoni, B. and B. Parukutty. 1979. Contiguous stomata in *Desmodium* Desv. (Papilionaceae). Current Sci. **48:** 27-28.
Pant, D.D. and P.F. Kidwai. 1966. Epidermal structure and ontogeny in some *Celatraceae.* New Phytol. **65:** 288-295.
Pant, D.D., and B. Mehra. 1964. Epidermal structure and development of stomata in *Ephedra foliata* Boiss. New Phytol. **63:** 91-95.
Patel, J.D., I.L. Kothari, and M.A. Pathan. 1972. Stomatal features in the leaf of *Aganosma dichotoma* (Roth) K. Schum. Ann. Bot. **36:** 849-856.
Patel, J. D. and J. J. Shah. 1971. Studies in stomata of chilli and brinjal. Ann. Bot. **35**: 1197-1203.
Payne, W.W. 1970. Helicocytic and allelocytic stomata: unrecognized patterns in the dicotyledonae. Amer. J. Bot. **57:** 140-147.
Stockey, R.A. and Dacrydium. 1992. Cutical micromorphology of *Dacrydium* (Podocarpaceae) from New Caledonia. Bot. Gazette **151:** 138-149.
Stockey, R.A., B.J. Frevel, and P. Woltz. 1998. Cutical micromorphology of *Podocarpus*, subgenus *Podocarpus*, section *Scytopodium* (Podocarpaceae) of Madagascar and South Africa. Int. J. Plant Sci. **159:** 923-940.
Su, J. X., F. Liao, and Y.Y. Huang. 2003. The leaf epidermis of three species in *Zamiaceae.* Acta Bot. Yunn. **25:** 596-602.
Sun, T.X., Y.S. Hu, and K.Y. Lang. 1999. A study on micromorphological characters of leaf epidermis of *Neottianthe* in China. Acta Bot. Yunn. **21:** 57-62.
Sun, T.X., S. Zhao, and X.Y. Zhuang. 2001. Leaf epidermal structure in 10 species of Annonaceae. J. Trop. Subt. Bot. **9:** 194-200.
Tang, M., Y.X. Hu, J.X. Lin, and X.B. Jin. 2002. Developmental mechanism and distribution pattern of stomatal clusters in *Begonia peltatifolia*. Acta Bot. Sin. **44:** 384-390.
Torrey, J.G. and H. Berg. 1988. Some morphological features for generic characterization among the *Casuarinaceae*. Amer. J. Bot. **75:** 295-301.
Xi, M.L., S.K. Bao, and D.D. Lin. 1997 The epidermal structure of *Camptotheca acuminata* leaf. J. South west China Normal Univ. (Natural Science) **22:** 58-61.
Zhao, X.Z., Y.S. Yang, and Z.X. Shen. 2006. Stomatal clustering in *Cinnamomum camphora*. South Africa. J. Bot. **72:** 565-569.
Zheng, Y. and J. Gong. 1999. A leaf epidermis study on tweleve species of sedum in AnHui. Bull. Bot. Res. **19:** 292-297.

氣孔成簇現象，陸生植物感受和適應環境的新表徵

甘 毅 周 磊 沈仲佶 沈竹夏 張一瓊 王根軒

中國浙江大學 生命科學學院農業生態與工程研究所

“氣孔簇”是一種由二到多個氣孔組成的非正常氣孔排布格局，目前在 60 多種陸生植物上已有報導。根據它們的形態特徵和分佈格局，又可劃分為兩種不同的氣孔簇類型。然而，通過對 16 種植物葉表皮的氣孔分佈格局進行分析發現，經典生態學上的空間分佈 R 係數不能很好的區分上述兩種氣孔簇。因此，為了更好的區分它們，本文引入了“接觸型氣孔簇”和“非接觸型氣孔簇”的概念，並且分別討論了它們的形成機理和潛在的生態意義。為了探究氣孔簇的形成是否與環境脅迫有關，蠶豆幼苗被種植在不同的水分和鹽分梯度下。兩周後，撕去葉表皮條進行氣孔格局分析。結果表明：乾旱和鹽脅迫明顯增加了蠶豆葉片的氣孔密度和氣孔指數。而且接觸型氣孔簇的出現頻率也明显增加。這個結果暗示著氣孔簇的出現與外界環境信號有關，它們可以作為陸生植物感受和適應環境的新標誌。

關鍵詞：氣孔簇；一細胞間隔法則；環境脅迫；氣孔發育；蠶豆。