Clustered Stomates in *Begonia:*

An Exercise in Data Collection & Statistical Analysis of Biological Space

JOANN M. LAU ROBERT W. KORN

An important aspect of biology is **spatial distribution** and patterns of distribution can be either ordered, random, or clustered. For examples, deployment of mature trees in a forest is ordered, occurrence of zebras on a savannah is clustered, and location of clams on the ocean floor is random. Deployment can be examined in two directions; first is down the scale of order to study arrangement of organisms (trees, zebras, trout) in a population, leaves on a branch, **stomates on a leaf**, chloroplasts in a cell, etc. The second approach to the study of deployment is to provide a quantitative measure of a pattern. One means of quantifying space is by the dispersion index, DI, which is the variance over the mean, σ^2/m; a value greater than 1.0 indicates an ordered arrangement, a value of about 1.0 denotes a random pattern, and a DI noticeably less than 1.0 comes from clustering.

An interesting example of all three patterns is the occurrence of clustered stomates on leaves in some *Begonia* cultivars (Figure 1). In this figure stomates are found in clusters and the arrangement of these clusters is indicated by the DI which is 0.64/3.25, or 0.19, much less than 1.0. Also notice that clusters are usually separated by one cell, again, a relation that gives an ordered pattern. For the arrangement of stomates the DI is 74/13, or 5.7, much greater than 1.0 and so the pattern is one of order. Finally, the number of stomates per cluster has a DI of 3.1/3.6 (Table 1), or 0.87 close to 1.0 for a random number of stomates per cluster.

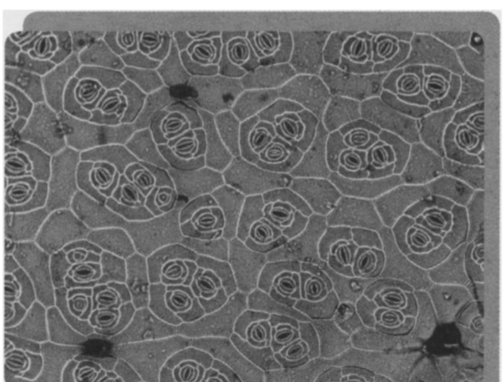

Figure 1. A fingernail polish imprint of a field of stomatal clusters of *Begonia* X *semperflorens* "Whiskey" with clusters ranging from one to five (zero to four additional stomates). Notice that the distance in cell number between a cell and its closest neighbor is nearly always one cell. Bar is 200 µm.

Data collection and statistical analysis of the data is the hallmark of modern science but few teaching exercises are designed for students to experience this approach. The difficulty with handling data is that it requires either too many organisms or the technological requirements are unrealistic. For a class to study the spatial distribution of trees, animals in a herd, or clams on the sea floor is unrealistic both with respect to travel and to have sufficient numbers to make sound conclusions. One example where many data points can be collected with minimal equipment is that of stomates (Greek for "openings") on the leaves of plants. In some cultivars of *Begonia,* stomates occur in clusters ranging from one to as many as ten (Payne, 1970) and these can be seen by a permanent preparation of a microscope slide as a fingernail polish imprint (Sampson, 1961) as shown in Figure 1. Seen under the microscope, 100 clusters can be counted for the number of stomates per cluster in only 15 minutes and, if desired, 500 within an hour. This data set can then be analyzed statistically by calculating the average as well as variance and then tested to see if they fit a particular expected distribution, in this case the Poisson distribution. The exercise can be done in middle school classes by students making their own slides and seeing imprints of cells, or at the high school level through collecting data of number of stomates per cluster and graphing the results, or in a college laboratory by comparing collected data to an expected Poisson distribution and doing a χ^2 test for goodness of fit.

JOANN M. LAU is a graduate student in the Department of Natural Resources and Environmental Sciences at University of Illinois, Urbana, IL 61801. ROBERT W. KORN is Professor Emeritus at Bellarmine University, Louisville, KY 40205; e-mail: rkorn@bellarmine.edu.

Procedures

Begonia (*B.* X *semprflorens*) plants can be purchased in the spring at a local nursery or are grown indoors year round. Some cultivars have stomatal clusters, such as "Vodka," "Whiskey," or "Lady Carol," while others, *B.* X *tuberhybrid*, have the more typical solitary stomates, so plants have to be checked well before class. A piece of leaf about the size of a postage stamp is cut off the plant and the imprinting method of Sampson (1961) is run as follows. The lower epidermis is swabbed with clear fingernail polish, left to dry for about 15 minutes, and then peeled off with a pair of forceps. This imprint is then placed on a clean microscope slide, a coverslip is placed over it, and a small dab of the fingernail polish is placed at two diagonal corners and allowed to dry in order to hold down the coverslip. This slide is placed on the microscope stage and observed at high power (~400X) as seen in Figure 1. Materials needed are listed in Table 2.

Table 1. Tabulation of *Begonia* data and calculations of the average (arithmetic mean) and variance of number of stomates per cluster.

A Number of stomates per cluster	B Number of additional stomates per cluster	C Number of clusters scored	D Total number of clusters scored	E B x D
1	0	I	1	0
2	1	𝍸 III	8	8
3	2	𝍸 𝍸 𝍸 𝍸	20	40
4	3	𝍸 𝍸 𝍸 𝍸 𝍸 II	27	81
5	4	𝍸 𝍸 𝍸 I	16	68
6	5	𝍸 𝍸 I	11	55
7	6	𝍸 III	8	48
8	7	𝍸 II	7	49
9	8	II	2	16
10	9		0	0
Total			100 (ΣD)	358

average (m) $=\Sigma E/\Sigma D = 358/100 = 3.58$

variance $\Sigma(B-m)^2/((\Sigma D-1) = 3.10$

The data are tabulated for 100 stomatal clusters as shown in Table 1. Column C is the number of additional stomates more than the first one and this step is important later in calculating the expected number of stomates per cluster. Next, the average, or mean (m) is determined as shown in Table 1. This step is done by a short method of adding up the products (B x D) of the number of additional stomates times the number of such clusters found and dividing by the total number of clusters scored, Σ(B x D)/ ΣD. The variance is calculated as the sum of the squares of the difference between each data point and the average divided by one less than the number of data points, or $\Sigma(x-m)^2/(n-1)$.

Table 2. Laboratory materials for preparing a slide of *Begonia* lower epidermis and for calculating average and variance of stomates per cluster.

- microscopes
- *Begonia* leaves
- blank slides
- coverslips
- clear nail polish
- forceps
- hand calculator (with y^x function)

First, the average and variance are calculated and are similar, 3.1 and 3.6, giving a DI value of 0.87 close to 1.0 as characteristic of a random distribution, notably the Poisson distribution. The Poisson distribution is a random distribution of the number of items in a small group, such as peanuts in a Hershey chocolate bar, points scored in a hockey game, or number of missed days of work in a year due to sickness. Next, the expected number of clusters of each type from 0 to 9 is calculated by doing a Poisson distribution. The Poisson equation is:

$$P(n) = m^n/n! \cdot e^{-m}$$

where *m* is the average calculated earlier, *e* is the base on the natural logarithms, or 2.718, and ! is the factorial (for example, 3! is 3 x 2 x 1 = 6 and 0! = 1 by convention). The calculations for the Poisson expectations are given in Table 3.

If the students are up to it, the statistical procedure of the chi square (χ^2) analysis can be made when comparing counted data and Poisson calculated data (Table 3, Column D) . There are then a sequence of four levels of study: slide observation, counting, Poisson calculation, and statistical analysis. The extent to which these are followed is at the discretion of the instructor.

Discussion

Students will study slides they made themselves rather than ones given to them and hence there is more motivation to do the tiresome task of data collection. To make this exercise more visual, graphs can be made plotting stomatal cluster size on the x-axis (abscissa) and the number of cases found on the y-axis (ordinate). Judith Croxdale, late of the University of Wisconsin, claims that fingernail polish stretches and becomes distorted, and suggests using Super Glue instead. Also, these slides are permanent so they can be looked at again at any time for a different study.

Another later study might involve the spacing of stomatal clusters (Korn, 1994). The distance between a cluster and the next closest cluster is measured in cell number, which is usually only one or two, never zero or more than two, thereby generating an ordered pattern like a checkerboard (Korn, 1994).

Finally, the exercise can lead to a discussion of the role of spatial arrangement in the life sciences. Students can be asked to think of other examples of a random distribution (ratio of boys to girls in a family of four children, freckles, etc.), an ordered distribution (placement of teeth and hairs), and a clustered deployment and size (herd, litter size). After this discussion, each student can be required to do a project by selecting a case of a distribution, collecting data, working out average and variance, and determining the type of deployment. This task puts students at the cutting edge of science in thinking about the spatial aspects of biological organization.

Since the techniques used here are relatively simple, it is not difficult for teachers to meet the *National Science Education Standards* on Inquiry and Life Science Concepts. Competence in the use of the microscope and handling minimal statistical procedures were probably common experiences in their college work as were exploring the concepts behind the use of simple biological statistics. However, the meaning of types of spatial arrangements may be more difficult if taken beyond the introductory level because of the mathematics required.

Table 3. Calculations for the expected number of stomates per cluster by the Poisson distribution.

A Number of additional stomates	B Number of clusters expected by the Poisson distribution $P(n) = s^n/n! \cdot e^{-s}$	C Obtained number of clusters counted (Table 2, Column D)	D χ^2 analysis $(B-C)^2/B$
0	$P(0) - 3.58^0/0! \cdot 2.718^{-3.6} \cdot 100 = 2.8$	1	1.1
1	$P(1) - 3.58^1/1! \cdot 2.718^{-3.6} \cdot 100 = 10.0$	8	0.4
2	$P(2) - 3.58^2/2! \cdot 2.718^{-3.6} \cdot 100 = 17.9$	20	0.2
3	$P(3) - 3.58^3/3! \cdot 2.718^{-3.6} \cdot 100 = 21.3$	27	1.5
4	$P(4) - 3.58^4/4! \cdot 2.718^{-3.6} \cdot 100 = 19.1$	16	0.3
5	$P(5) - 3.58^5/5! \cdot 2.718^{-3.6} \cdot 100 = 13.7$	11	0.5
6	$P(6) - 3.58^6/6! \cdot 2.718^{-3.6} \cdot 100 = 8.2$	8	0.005
7	$P(7) - 3.58^7/7! \cdot 2.718^{-3.6} \cdot 100 = 4.2$	7	0.8
8	$P(8) - 3.58^8/8! \cdot 2.718^{-3.6} \cdot 100 = 1.9$	2	0.005
9	$P(9) - 3.58^9/9! \cdot 2.718^{-3.6} \cdot 100 = 0.7$	0	0.7
		$\Sigma C = 100$	$\Sigma D = 5.51$

References

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