Alfalfa with n, 2n, and 4n gametes: Predicted and actual pollen diameter ratios

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Introduction

It is generally understood that there is a relationship between ploidy level and pollen size in alfalfa. In the early 1970s this relationship was used in studying diploids that produced *n* and 2*n* pollen based on crossing behavior. Close attention was not paid to diameter to volume relationships, and we later found that some plants had *n*, 2*n*, and 4*n* pollen. Materials were usually diploid populations that originally were hybrids of *Medicago sativa* × *M. falcata* (Bingham and McCoy, 1979). Research involving 2*n* gametes and sexual polyploidy involving 2*n* and 4*n* gametes was reviewed by McCoy and Bingham, 1988.

The objective of this report is to examine the diameter relationships of different ploidy levels of pollen in a tetraploid plant of *M. sativa-coerulea-falcata* origin that produces *n*, 2*n*, and 4*n* gametes. The respective pollen classes contain 16, 32, and 64 chromosomes, except for an unknown level of aneuploidy especially around the 16 chromosome class. The analysis emphasizes that a relatively small change in diameter results in a large change in volume.

Materials

As reference points, pollen sizes are reported for the following materials:

Clone '3' is a northern-adapted tetraploid alfalfa breeding stock; **3D** is an octoploid produced by treating **'3'** with colchicine.

KR6 is a hexaploid produced by an 8x-4x mating.

cv.com is a sample from an array of northern-adapted, tetraploid alfalfa cultivars.

The plant with *n*, 2*n*, and 4*n* pollen is referred to as Plant 5:

Plant 5 is from a complex hybrid of *M. sativa-coerulea-falcata*. **Plant 5** is one of 28 plants that were analyzed for reproductive abnormalities. Of the 28 plants, 7 had *n* + 2*n* pollen and 6 had *n* + 2*n* + 4*n* pollen.

Methods

Before starting to survey pollen diameters in order to detect gamete polyploidy, we used some simple algebra and geometry to predict the diameter ratios of pollen with *n*, 2*n*, 4*n* and other ploidy levels. Our proof follows:

Prediction of Radius Ratios for Pollen Grains of Different Ploidy Levels

Assumptions:

1. Pollen grains are spherical.
2. A regular, monoploid cell has **i** chromosomes. Adding **j** chromosomes increases the volume of a cell by (**j**/**i**) times, leaving the altered cell with a volume that is **k** times larger than the original cell, where **k**=1+(**j**/**i**).
3. Division spindle disorientation and/or failure increases the volume of the cell in multiples of **i**.

Solution:

$V_k = (4/3)\,\pi r_k^3$, where **V** is the volume of a sphere and **k** is the volume multiplier.

$V_k = kV_1 \rightarrow (4/3)\,\pi\, r_k^3 = k(4/3)\,\pi\, r_1^3 \rightarrow r_k^3 = kr_1^3 \rightarrow r_k = (\sqrt[3]{k})r_1$

And $2r_k = d_k$, where **d** is the diameter. This allows us to apply the radius multiplier to diameters as well.

Volume multiplier (k) :	Diameter/Radius multiplier ($\sqrt[3]{k}$) :	"Normal" ploidy:
0.25	0.63	
0.5	**0.79**	**mono**
0.75	0.91	
1	**1.00**	**di**
1.25	1.08	
1.5	**1.14**	**tri**
1.75	1.21	
2	**1.26**	**tetra**
2.5	**1.36**	**penta**
3	**1.44**	**hexa**
3.5	**1.52**	**hepta**
4	**1.59**	**octo**

Explanation:

These values can be scaled for different ploidy levels. When comparing diploid cells to monoploids or polyploids, the diploid is k=1, monoploid is k=0.5, tetraploid is k=2, hexaploid is k=3, etc. When comparing with another ploidy level as a base, the chart above can be scaled (for example, if tetraploid is k=1, then diploid is k=0.5, triploid is k=0.75, octoploid is k=2, etc.). As an example, let us take a plant with normal, *n* pollen grains that are approximately 1 unit in diameter. If the plant produces 2*n* pollen grains, we would predict them to be approximately 1.26 units in diameter.

Measuring and analyzing pollen sizes

In order to measure pollen, we tripped mature flowers onto new microscope slides. A drop of iodine in lactophenol was then added to the slide. After allowing the pollen to sit in solution for about a minute a cover slide was added and it was viewed under a light microscope. A random area densely covered with pollen was then photographed at 400x magnification with a 35mm camera using black and white professional-grade film. The photographs were then developed and increased in size by two times by photocopy. Because this study is unconcerned with the actual size of pollen and only with distribution of size and ratios, we were able to use blown-up photos for easier measurement. The pollen grains in the photos were measured using a millimeter ruler and the size was recorded in millimeters. The recorded size was then entered into a computer for analysis purposes.

After data collection, distributions of pollen sizes were viewed. These distributions were often in groups, with a very large group of the majority of pollen and two smaller groups.

Average sizes and ranges were calculated for each group. The average sizes of the assumed *n*, 2*n*, and 4*n* were then used to calculate an actual diameter ratio between each ploidy level. These ratios were then compared with the predicted diameter ratios.

Results

Average pollen sizes and ratios of tetraploid, hexaploid, and octoploid reference plants:

M. sativa	cv.com	Clone 3	KR6	3D
Avg	39.4	40.6	45.2	51.7
Ratio with cv.com	1.00	1.03	1.15	1.31
Predicted Ratio	1x = 1	1x = 1	1.5x = 1.14	2x = 1.26

Plant 5	n=39.5 mm (1x)	2n=50 (2x)	4n=62 (4x)
Estimated Actual	1.00	1.27	1.57
Predicted	1.00	1.26	1.59

Discussion

In the case of Plant 5 it was known from post-tetrad analysis that all four microspore nuclei were occasionally included in a single 4*n* pollen grain (Figs. 1, 2 and 3). Similarly, dyads were documented (Fig. 4) that were to become 2*n* pollen. The background of *n* and near *n* pollen grains provided a situation where theoretical and actual diameter/volume relationships could be studied. Reference plants also were available at tetraploid and octoploid levels and fitted expected diameter/volume ratios.

The eye easily detects the largest pollen grains in pollen analysis, making it appear that they are relatively frequent. In the sample photographed and analyzed, however, 4*n* grains occurred at a frequency of about 1:50, and 2*n* grains about quadruple that.

Examinations of file photos and photos in some publications indicates that pollen presumed to be *n* and 2*n* often actually contained *n*, 2*n*, and 4*n* pollen. The 2*n* pollen selectively functions in 4x-2x and 8x-4x crosses and the 4*n* pollen functions only in special cases (see the review). Hence, the occurrence of 4*n* pollen usually does not affect progeny recovered, but it is important for a basic understanding of reproductive mechanisms.

This pollen analysis is also handy for looking at gametogenic abnormalities that may occur in a plant that is known to be of a certain ploidy level and for discovering the frequency of gamete ploidy level abnormalities in a certain plant. It is also possible that this approach could be expanded to somatic cells.

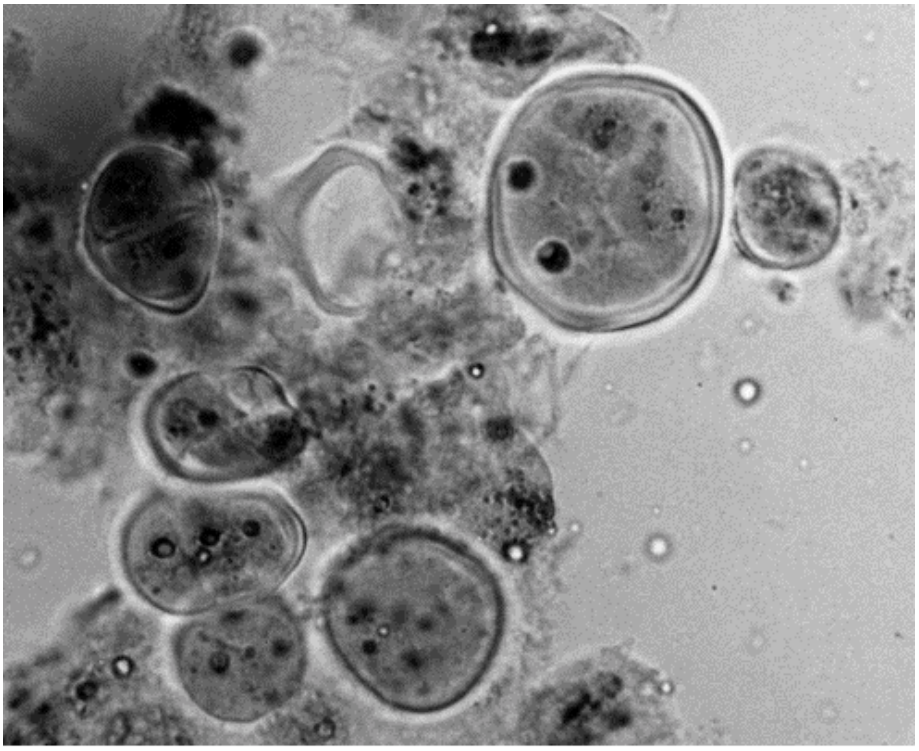


Fig. 1: A 4n microspore surrounded by n and 2n microspores in Plant 5.

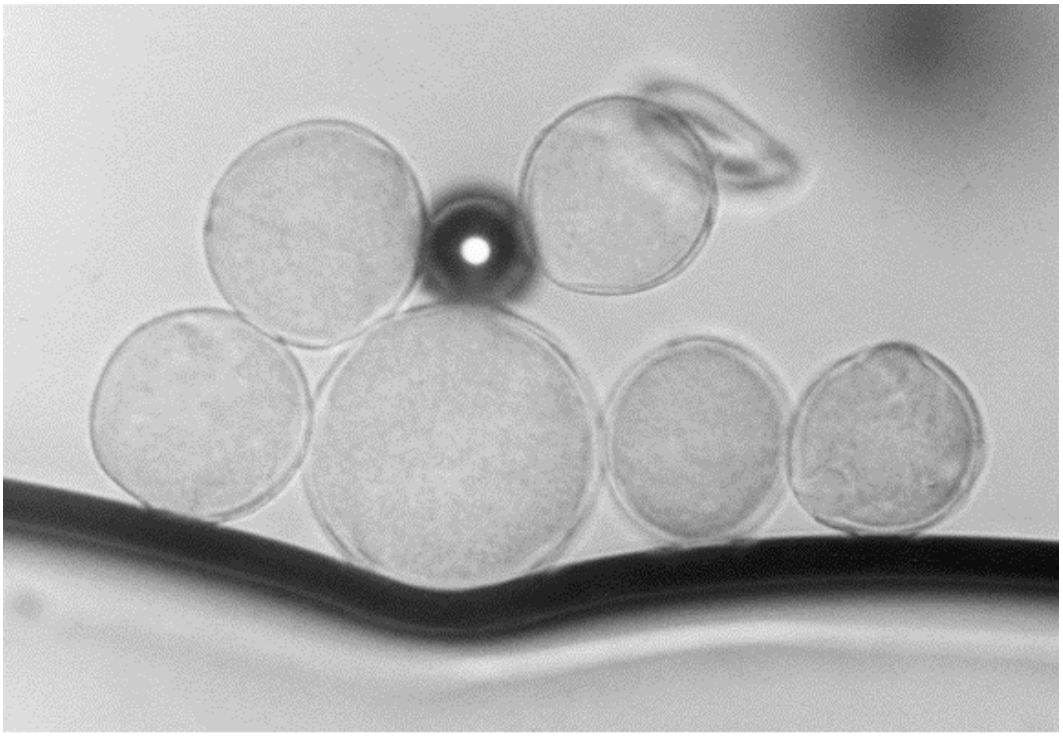


Fig. 2: Example of n, 2n, and 4n pollen in Plant 5.

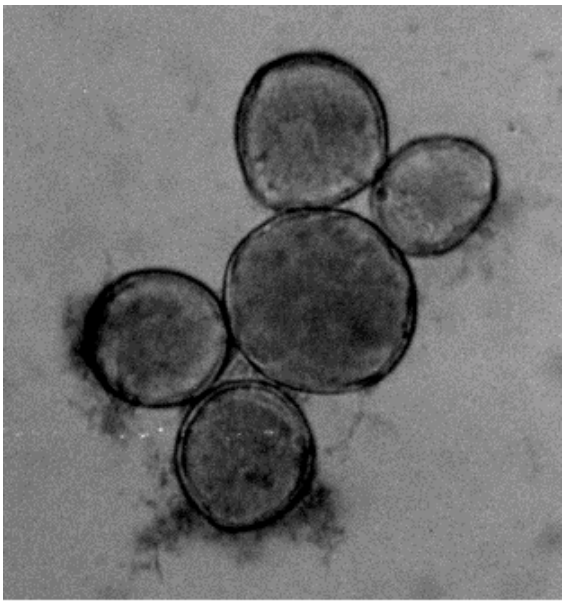


Fig. 3: Example of n, 2n, and 4n pollen in Plant 5.

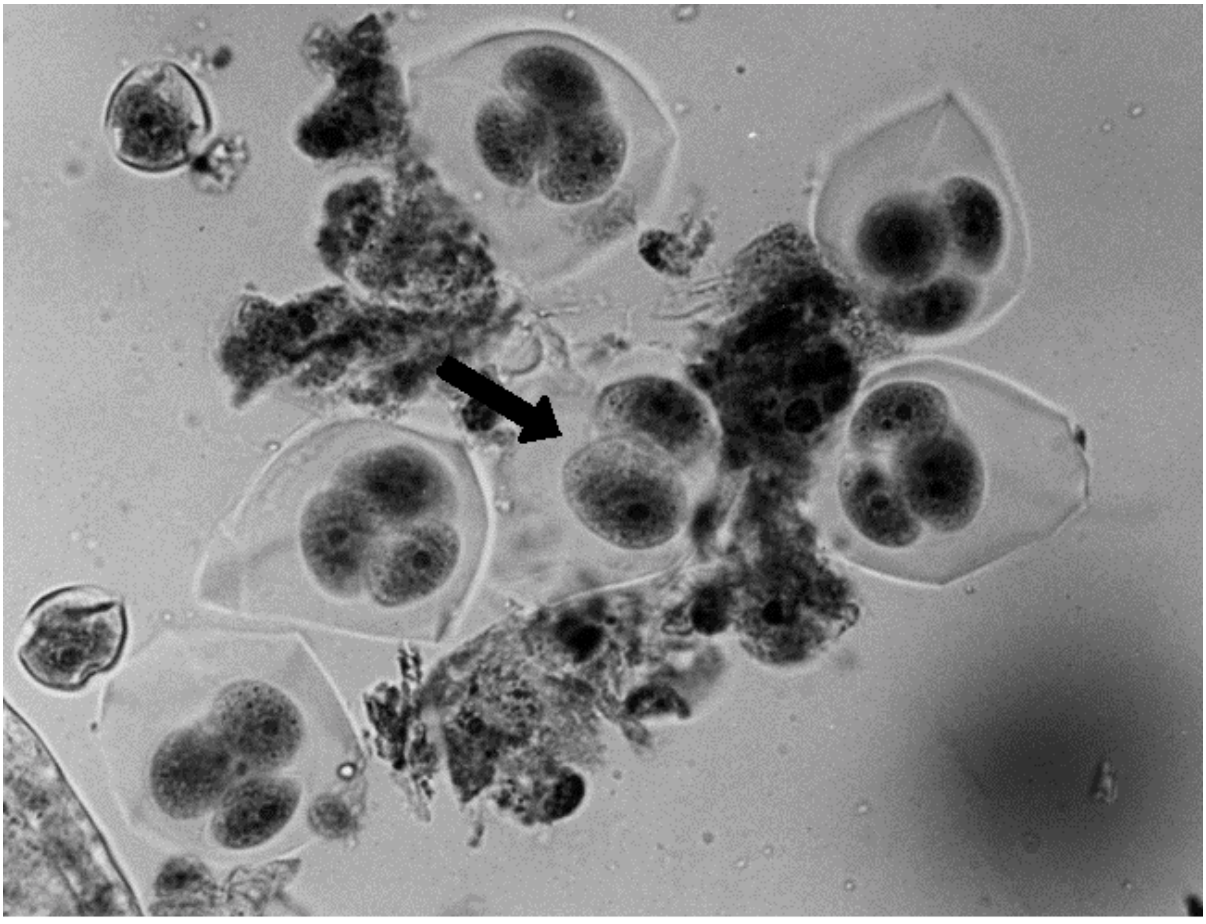
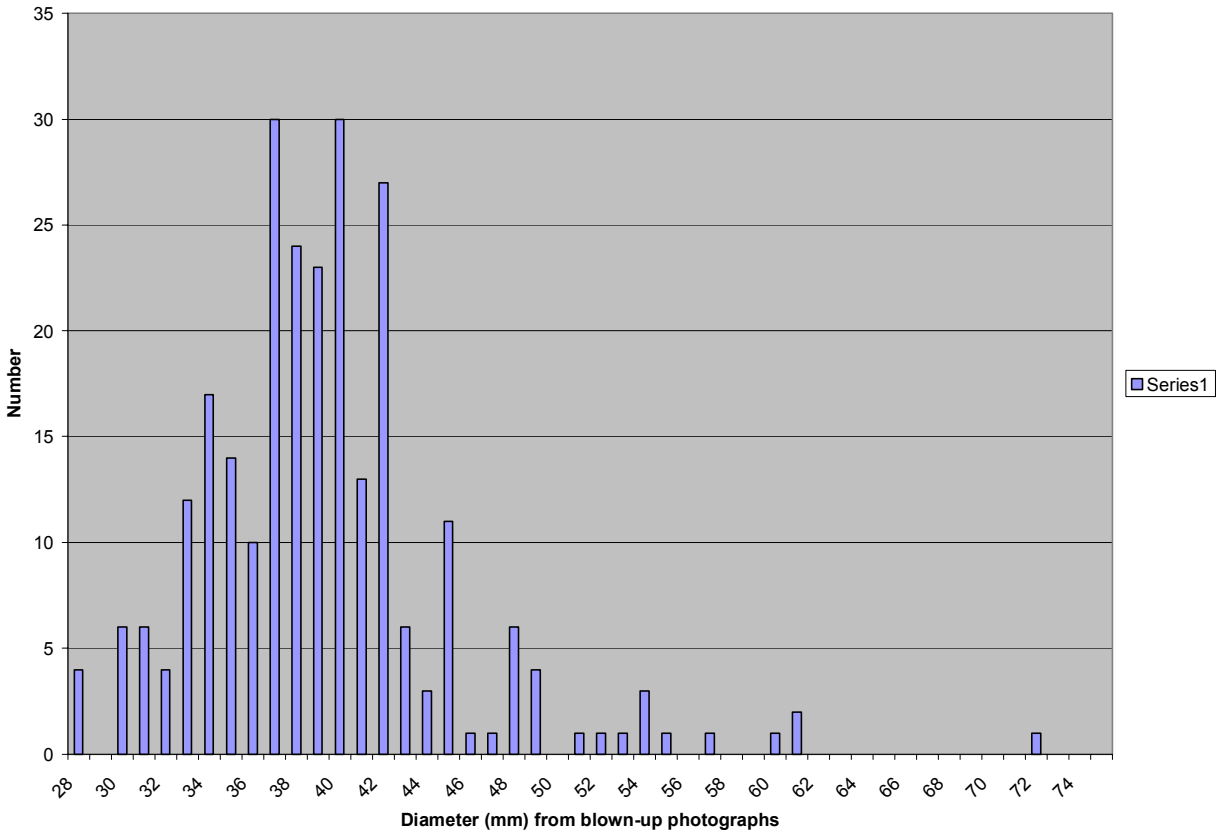


Fig. 4: Tetrads and one dyad (arrow) at end of meiosis in Plant 5.

Fig. 5: Plant 5 Pollen Diameters



Bibliography containing additional references

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