

PAEONIA

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TO: Chris Laning  
FROM: Larry L. Harder, P.O. Box 278, Ponca, NE 68770  
DATE: July 31, 1987

Dear Chris:

I do have a few seedlings that I like, but feel that they haven't been established long enough for me to tell if they are really all that great yet. This year I tried to germinate some of the seeds from the tree peonies. Got a couple up, but then forgot about them, and put them outside — and before I remembered, they had dried out too much so I lost them. Maybe I'll have a few seed this year to try again.

Always was going to send for a few seeds, but never did. Now that I've started a garden out on the farm, I guess I'll have the room to plant out a row or two. Have a row here in town that are starting to bloom. Nothing real exciting in them yet. Hopefully will find at least one that I'll like.

Have been using some of the yellows, but they must be very recessive. Will see what I get from the seedlings when they are backcrossed.

Have been doing too many irises, and now daylilies also, to get really into peonies.

Best wishes,

Larry

## HYBRIDIZING

It has been stated that about 3% of a group of seedlings will be found to be superior at least in some respect, thus making progress rather slow. This, then, supposes that you "must have population". Added to this proposition is the worrisome fact that not all desirable features show up in the juvenile stage. This indicates that evaluating must take place over a number of years. Selecting and roguing can be disastrous to long-range progress if done on young seedlings. Along with this is the fact that goals are arbitrary at best and change oftentimes. A discarded seedling cannot be brought back to life. — But you can't keep all of them!!, so what to do???

Mr. Brand was successful in introducing many excellent lactifloras. To follow his example would mean raising seedlings by the acre: And that means lots of work and a large investment.

Several lines, based upon parentages, are being raised such as 'Moonrise' x 'Archangel', Quad F3, Quad F3 x 'Silver Dawn' F3, 'Sable' x Super D", etc. All of these (22 lines of breeding) have produced an abundance of seeds, far too many to plant, so only a few from each line will be planted, the rest have been thrown together and for distribution will be called tetraploid mix. Along with the tetraploid lines are two lactiflora strains! Eugene Screwball, a plant gotten from Catherine Brown of Pennsylvania, that has distorted flowers; and Water Soaked from Roy Pehrson, which has black red flowers.

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For hybridists who have limited areas (acres) and time, there are three lines that have only limited competition and offer wonderful opportunities. These are lutea hybrids, suffruticosa (T.P.), and Itohs. The Itoh line offers the best opportunity for superior landscape plants with excellent flowers.

The lutea hybrids are in short supply and not well known so the field is open to all of us.

The tree peony, suffruticosa, is the easiest of the three and has the widest range of colors and textures and size. A large selection of clones with American names is needed to run alongside the European and Japanese introductions. The most difficult feature in raising T.P.s is the waiting for little seedlings to mature.

Roger Anderson is doing great things with the Itoh cross and has already registered several. The color range of his introductions caught us by surprise! We thought all Itohs would be yellow — not so! Also, he has discovered a lactiflora called Martha W. is a superior pod parent (seed parent) and that pollen from Dr. Reath's lutea hybrid #199 make a great couple!

LETTER FROM DARLENE S. TYLER  
1917 Walnut Bottom Rd.  
Carlisle, PA 17013

DATE: February 21, 1987

Dear Chris,

Enclosed is a check for \$2.50 to cover my contribution to PAEONIA.

PAEONIA is an important publication to me, a new hybridizer, since it helps gather information and ideas about hybridizing. Information about good hybridizing cultivars, what you are trying to accomplish as a hybridizer, your success in breeding in or out a characteristic, which characteristics are dominant and which are recessive and how you have coped with them all help to get my hybridizing ideas mobilized.

I've collected my first crop of seeds this year – it was much smaller than I expected! – and on to flowers a few years down the road!

Can you consider developing a more scientific format for the peony registration list? If the information were arranged in the same fashion and the same information were given for each cultivar, it would be easier to use to find flowers with specific characteristics.

Have a good year.

Sincerely,

Darlene S. Tyler

ED. NOTE:

It seems that what Darlene S. Tyler wants is specific information on what to do and how to do it. To my way of thinking, hybridizing is an art more than a science. If there are facts in this game, I don't know them.

Dominant and recessive are relative terms – dominant to what, and recessive to what? In a cross you may make, you expect something but get something else!

A scientific format for peonies being registered is still beyond our capability. I don't suppose anyone knows the genetic content of any cultivar, and anything less than tabulation of genetic content eliminates the scientific category.

You are asking far too much!

- Chris

The Use of Indirect Ploidy Indicators as an aid in the Preliminary  
Identification of Colchicine Induced *P. suffruticosa* Tetraploids

L.J. Dewey, 2617 Wyndham Drive, Richmond, VA 23235

June 1987

Summary

A *Paeonia suffruticosa* seedling whose epicotyl was treated with 0.45% aqueous colchicine in May of 1981 flowered for the first time in April of 1986. The seedling exhibits somewhat above average vigor compared with untreated seedlings and it tends to have darker green leaves. The seedling produces pollen whose average size is larger than that produced by control plants. When selfed, the seedling produces seed which are significantly larger (over 75 percent) in weight and volume. Limited fertility tests did not show a clear-cut increase in fertility compared with controls. The increase in pollen and seed size is taken as an indication that the aerial portion of this seedling is tetraploid.

Since 1977 I have been treating the epicotyls of tree peony seedlings with colchicine in an attempt to induce tetraploidy. The method of treatment is a modification of the procedure described by Reath (APS Bulletin #201, March 1972, pp 13-15; reprinted in "The Best of 75 Years," APS, 1979, pp 153-154) and was reported earlier (*Paeonia* 9 (4), 2-3, December 1978). Since that report, I have settled on using a 0.45% aqueous colchicine solution in the treatment. Even so the survival rate of the seedlings is probably less than 50% and it was not until last spring (1986) that the first treated seedling bloomed.

Once a group of colchicine treated tree peonies has been established, how can a tetraploid plant be identified among the seedlings? The obvious answer is to examine the plant cytologically and count the chromosomes. However, this is a specialized technique for which I lack the training and equipment and could ultimately involve the examination of a fair number of plants. Moreover, a root tip count of the chromosomes of these seedlings could be inconclusive since presumably the root systems are diploid. As a result, I have been forced to look at some indirect ploidy indicators in my attempt to identify likely tetraploids among the treated *suffruticosa* seedlings.

Dr. Mark Cathey, former president of the American Horticultural Society, once enumerated some of these indicators in an interview with Bernard Collier published in the popular newspaper magazine, "Parade," March 18, 1979 and of course there were numerous references to them in the scientific literature. Some of the plant characters which have been used are listed below.

1. Increased vigor, including larger, thicker, darker green leaves, larger stems, larger roots.
2. Enlarged stomatal guard cells.
3. Larger pollen.
4. Larger seed.
5. Fertility may be increased.

It might be instructive to consider some of these indicators as they apply to *suffruticosa* seedlings. As a result of the doubling of the chromosomes from the diploid to tetraploid condition, the size of the individual cells can be expected to increase. This in turn could affect the appearance of the vegetative growth in that the plant may exhibit increased vigor, larger, thicker and darker leaves, larger stems and larger roots. These traits are difficult to measure

quantitatively in peonies although they have been used as indicators of polyploidy in other genera. I have found so much variation in diploid suffruticosa seedlings growing in my garden that it is not easy to establish normal control levels for these traits. Part of the problem is that the tree peonies in the diploid state tend to be vigorous growers and have large leaves, and any increase in these characters, unless it is dramatic, is not so easily discernible.

Many tetraploids exhibit an increase in the size of their leaf stomata. I have not examined this characteristic in peonies because it requires special leaf tissue preparation and such careful microscopic measurements. Moreover, in other genera the size increases are not always clear-cut. If the stomata size were to double, this could be a very helpful indicator and I still may look at peony stomata in the future if I can master the technique.

Three other indicators of polyploidy, suggested in the literature, are increases in pollen size, seed size and fertility. An increase in fertility is not always easy to establish. Furthermore fertility may actually decrease in some induced tetraploids if aberrations occur during meiosis. These three indicators are the main subject of this report.

#### Description of Seedling

The first colchicine treated suffruticosa seedling to bloom for me had its maiden bloom in April 1986. This seedling was grown from a mixture of Japanese tree peony seeds supplied by T. Domoto and was assigned the number C252-81. The seedling epicotyl was treated with 0.45% aqueous colchicine in May of 1981 according to the method cited above. Since only the epicotyl region of this seedling, and not the root system, was treated, the present root system presumably is diploid. Even if the aerial portion of the plant is tetraploid, the diploid roots may affect the expression of tetraploid characteristics in the upper plant. As a result there may be some suppression of plant vigor and leaf size and leaf thickness because of the diploid root system. This seedling exhibits somewhat above average vigor compared with untreated seedlings from the same lot and it tends to have darker green leaves. The single flower (10 petals) was a deep pink becoming lighter toward the petal edges and measured slightly over eight inches across. It had sharply defined flares of a medium magenta color. The red-sheath entirely covered the carpels which carried dark magenta stigmas.

This seedling was transplanted in the fall of 1986 and did not bloom in the spring of 1987. However, another colchicine treated seedling (C216-81) from a different seed lot had its maiden bloom this spring (1987). Both of these seedlings, which were treated with colchicine in 1981, have bloomed at an earlier age than the untreated controls, none of which has bloomed yet. Whether or not this observation has any significance remains to be seen.

#### Fertility

As yet I do not have much data on the fertility of this seedling (C252-81) and what I have points to a low level of fertility. The maiden flower was selfed in April 1986 and produced five seeds which will be discussed in more detail later. Incidentally, using the indoor germination method these seeds are now beginning to produce roots in the damp vermiculite and, assuming the aerial portion of the parent is tetraploid, should produce completely tetraploid seedlings. Pollen from the flower was used to make a number of protected crosses which are summarized in the Table below.

### Crosses Made with C252-81 Pollen

Pod Parent Types	Num. of Varieties Crossed	Num. of flowers Crossed	Num. of Seeds Produced
Lutea Hybrid F1	8	13	2
Lutea Hybrid Advanced Generation	1	1	0
P. lutea	3 clones	4	0
P. lactiflora	3	6	0
Herbaceous Hybrid	1	1	0
Itoh	3	3	0

As can be seen from the table only two seeds were produced out of 28 flowers pollinated. The successful crosses were as follows:

**'Age of Gold'** x C252-81      1 seed  
 TP1-66 x C252-81              1 seed  
 (TP1-66 = full double, yellow, unknown lutea hybrid Japanese import; may be **'Alice Harding'**.)

The rooted seedlings from these two crosses are now being chilled in the refrigerator. Granted that most of these crosses are difficult to make, the data still do not say much for the fertility of this seedling. No crosses on suffruticosa varieties were made in the spring of 1986 but a few were made this spring (1987) using stored pollen from the seedling. The results of these crosses will have to wait on the seed harvest this fall.

#### Pollen Size

Increased pollen size is another characteristic which may distinguish tetraploid plants from their diploid counterparts. In a series of elegant electron micrographs, Maynard Dewey (A.P.S. Bull. No. 259, Sept, 1986, p12) has given us a glimpse of the structure of the peony pollen grain. When viewed on the side, the grain is reminiscent of a football or a grain of wheat, but when viewed on end, it is seen to be deeply furrowed into three segments. Considering the structure of peony pollen it would obviously be more accurate to measure the volume and/or weight of the grains when comparing sizes. However, although measurements of this sort may be possible, they certainly would be tedious with these microscopic structures. An indirect approach would be to treat the grains as flat microscopic particles and in effect make measurements in only two dimensions. This is what I have done. Although we cannot calculate the volume from these measurements, the measurements should be at least roughly proportional to the volume.

Using a Quantimet 970 Image Analysis System (Cambridge Instruments), pollen grains from the seedling in question and from several different suffruticosa garden varieties have been measured. Data from the field of the system's microscope are electronically transferred to a computer where the data are processed to give the area, perimeter, width and length of particles in the microscope's field. Measurements were made on several pollen grains from each variety and the results were averaged for each variety. These averaged values along with the variety designations are shown in the table below.

**Comparison of Average Dimensions of Single Pollen Grain  
of Suffruticosa Garden Varieties with Seedling C252-81**

Variety	Number of Grains	Length (microns)	Width (microns)	Perimeter (microns)	Area (square microns)
TP1-75	8	75.9	40.2	194.4	2379.2
TP2-75	5	78.0	37.4	193.0	2285.0
TP6-78	5	82.4	41.6	209.2	2584.4
TP1-82	5	83.6	43.8	215.6	2872.4
Avg. of above 4 varieties	23	80.0	40.8	203.1	2530.2
Avg. of 1st 3 varieties	18	78.8	39.7	198.9	2416.2
C252-81	11	91.8	45.6	226.2	3180.5

*Note: There are over 25,000 microns in an inch.*

A comparison of the overall averages for the four suffruticosa garden varieties with those of the seedling shows that the seedling had larger values for all parameters measured. If we exclude the TP1-82 data, whose values are closest to those of the seedling, from the averages and compare the differences and compare the averaged values for the first three varieties with those of the seedling, the differences are even more striking. TP1-82 is an interesting unknown variety imported from Japan and has a flower resembling that of Rock's variety except the flower is double. If it is related to Rock's variety, it may have larger pollen than the ordinary suffruticosa garden varieties since Maynard Dewey has reported, in a private communication, that in his study cited above, he found an average length of 87.6 microns and an average width of 48.3 microns for pollen from Rock's variety. These values are much closer to those of TP1-82 and the seedling than they are to the averaged values from the first three varieties shown in the table. These results complicate the interpretation of the data on pollen size but I still feel the large size of the seedling pollen may be taken as an indication that the aerial portion of the seedling is tetraploid. It is obvious that more data is needed on the size of suffruticosa pollens.

Seed Size

Finally, the last ploidy indicator I want to discuss is seed size. As indicated earlier, the maiden bloom of this seedling was selfed and bagged to protect it from cross pollination. The plant produced five seeds which are the largest suffruticosa seeds I have ever seen. The seeds were air-dried and then weighed. For comparison air-dried seeds from five suffruticosa garden varieties were also weighed. From these data the average weight per seed from the seedling and from the varieties was calculated and the results are shown in the table below.

After weighing, each lot of seeds was immersed in a known volume of water in a graduated cylinder. When the seeds sank below the surface, the volume of water displaced by the seeds was measured. Using this volume, the average volume per seed was calculated for each variety and these results are also shown in the table below.

**Comparison of Average Weights and Volumes of Single Seeds  
of suffruticosa Garden Varieties and Seedling C252-81**

Variety	Number of Seeds	Weight (grams)	Volume (mL)	Density (g/mL)
Haku Jin (Reath)	22	0.474	0.45	1.064
TP1-75	29	0.339	0.31	1.106
Higure (Reath)	35	0.400	0.37	1.094
TP2-76	12	0.342	0.29	1.174
Yae Zakura (Reath)	21	0.275	0.26	1.049
Overall Average		0.366	0.34	1.097
C252-81	5	0.651	0.60	1.085

From the data on the average weights and volumes of individual seeds shown in the table, it can be seen that the seeds from the seedling are substantially larger than those from the suffruticosa garden varieties. In the case of seed weight, the C252-81 seeds are about 78% heavier than the average garden variety seed while the volume of the seeds from the seedling is about 76% larger than the average garden variety. Of all ploidy indicators examined for this seedling, the increase in seed size is the strongest evidence that the aerial portion of the C252-81 seedling is tetraploid.

The densities of the seeds, calculated from the weights and volumes, are shown in the last column of the table. The average densities of the seeds from the varieties varies from 1.049 to 1.174 with an overall average of 1.097. This is a fairly narrow range and apparently has no bearing on the ploidy of the seed since the density of the C252-81 seed is close to the average value. In view of the fact that the density of water at room temperature is slightly less than 1, the seeds will sink when placed in water.

Conclusions

Although several of the ploidy indicators investigated for seedling C252-81 gave equivocal results, the marginal increase in pollen size and the substantial increase in seed size are taken as indications that at least the aerial portion of this seedling is tetraploid. This conclusion is in agreement with the one reached by Reath (reference cited above) that tetraploidy can be induced in peonies by treatment with colchicine. These preliminary results would seem to make it worthwhile to attempt to get the tetraploid portion of this seedling on its own root system by grafting or layering so that chromosome counts could be made on the root tips.

## CORRECTIONS REGARDING "SMOUTHII" – "LACINIATA"

Nancy Ann Halas

Page 111 of Stern's monograph contains an error perpetuated as a truism ever since his publication a great many years ago. Stern was good enough to compile a collection of writings on the Peony from many sources, some true, some fanciful, some downright incorrect. This is not a bible of sorts, it is however worth reading and studying because there isn't that much available in Peony literature and you have to use whatever resources are available.

Stern cites Horticulture Universal, vol. 4, 247 (1843) as saying that P x smouthii is a hybrid between P. Albiflora and P. Tenuifolia.; this plant is sometimes listed in nurserymen's catalogs as P. Lacinata. The article goes on to say that this hybrid may have been called after Monsieur Smout, a chemist in Malines, who was a keen hybridizer of plants.

The characteristics of the plant is such that the flowers are nodding, a maroon typish color, with attractive leaves that resemble both tenuifolia and anomala. The point is that the nodding flower characteristic resembles the anomala species series. The softness of the flower stem causes the nodding characteristic of the flower which is attractive in its own way.

Some other examples of the nodding flower transmission in hybrids is found in the Windflower series by Saunders. The cross P. Emodi and Vietchi is white with nodding flowers and is called Early Windflower. The Windflower Late is Anomala by P. Emodi. This too had nodding flowers. The color is mostly taken over by the species other than Anomala. P. anomala transmits the nodding flower characteristic of windflower term as Saunders preferred to point it out.

What I am pointing out is that Lacinata or Smouthii as it is or may be known, is not a hybrid of Albiflora but is correctly a hybrid of both Anomala and Tenuifolia, and the flower will nod to agree. It is pretty for landscaping because of the pretty leaves and I would rather adopt this as a specie for Michigani, since we don't have a native peony anyway and it will grow here.

For those of you hybridization buffs, I am providing you with an opportunity to verify the disclosure, but I think that many of you will agree even at this point. The species anomala transmits the nodding the flower characteristic to hybrids, and this is an attractive feature really. It is however a characteristic that Albiflora does not do. For example when P. Alice Harding was crossed with Albiflora, the stems changed from drooping to stiff and erect, in the Itoh series as we now know them.

In the Anomala series, I believe that the proper characteristics are nodding flowers and some color purple or mauve flowers. The one called Intermedia is a little suspicious to me since it calls out a bright red flower, which is uncharacteristic of the Anomala series. I don't know if the flowers are nodding or not since this is one plant I don't have in our collection, although I thought that we did have it at first. We do have some bright red flowered plants with cut leaves, but these are erect flowers and not nodding and so I would characterize that one as a hybrid of Albiflora and Tenuifolia.

Peonies are fun to hybridize as many readers like to do as a hobby and form of entertainment. We do have enough history on many of them to forecast what the offspring should look like in some cases. There are obvious errors that do crop up and we have to acknowledge that nobody is perfect and mistakes are on occasion published even in prominent peony handbooks particularly since there is never any revision of them. However we have to continue on.

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Editor's Note:

Nancy sent along with this article three sheets of pictures of leaf structure which support her proposition. For those of you hybridisers that use these plants in your program, I can send copies of them. But to include them in PAEONIA would add too much in postage.

- Chris

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NOTICE:

Bill Seidl is in charge of the Seed Distribution program. A listing of seeds that will be available should be found in the American Peony Society Bulletin, December issue.