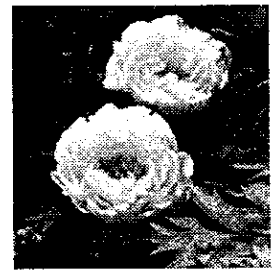


# PÆONIA



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## POLLEN VIABILITY

by Don Smith

Maintaining the viability of pollen samples is a critical element in having a successful breeding program, especially when attempting difficult crosses. Saunders is reported to have found that pollen remained viable, if kept in the open in a dry room, for over fifty days (The Peonies, p. 121). However, this may not be true under all conditions. On several occasions in various articles I have commented on ways of maintaining pollen viability. I have consistently recommended that pollen be kept in a refrigerator when not in use to prolong its viability. This recommendation was based on various articles that I had seen over the years as well as from my own experience working with

peonies. Recently, while searching through my files looking for something else, I came across one of these articles on pollen viability. Although this study was not conducted with peonies, I believe that the results of this work can be generalized to other plants with tri-celled pollen including peonies. Therefore, I have tried to briefly summarize this article below.

A study by C. Dumas et al. (1983) found that pollen viability in *Brassica napus* (rape-seed plants) went from 90% to 0% in just 12 days when the pollen was kept at room temperature (20-23°C). These authors also concluded that the loss of viability was correlated with the loss of water content during the pollen grain aging. Pollen viability was found to decrease fairly rapidly during the first day, then much more gradually for the next 3-4 days, then rapidly again thereafter. Viability was found to be approximately 75% after one day, about 50%

after one week, ~25% at 10 days and 0% after only 12 days (see figure 1 below). However, it was also determined that a certain residual amount of water persisted even after the pollen viability reached 0%.

this purpose. They are more than large enough to hold all the pollen that could ever be used in one season, yet small enough so that several dozen "cans" require only minimal space in the refrigerator. Most important of all, these

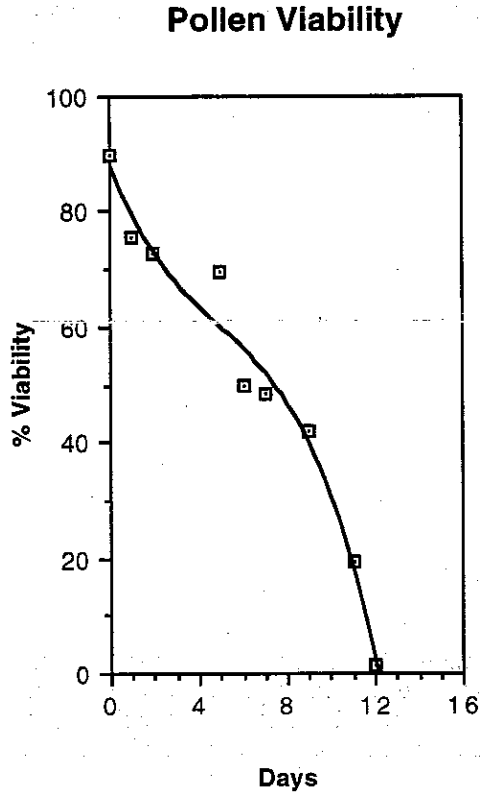


Figure 1

In addition, these authors found that the weight loss rate (i.e., water loss) increased rapidly with increasing temperature. Water loss was found to be more than 10 times faster at 90° F compared to 72° F and almost 15X faster still at 108° F compared with the 90° rate. These data are shown in figure 2. Extrapolation of these data down to normal refrigerator temperatures (34-38 °F) indicates that pollen water loss in a refrigerator would be about 100X slower than that at normal room temperatures (68-72 °F).

I always store my pollen in black 35 mm film canisters. These small plastic "cans" are readily available, easy to label and are the perfect size for

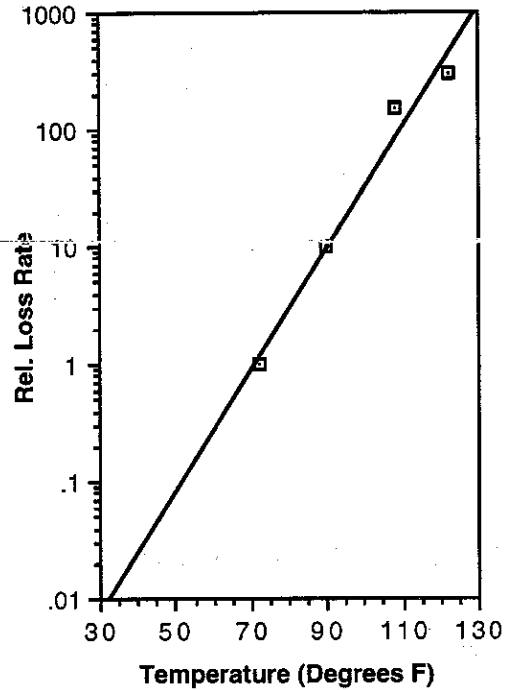


Figure 2

containers are also air-tight and completely moisture-proof. These "cans" should always be kept in a refrigerator or freezer whenever they are not being used in the garden. I usually transport my pollen and other supplies to and from the garden in a small portable cooler. It is especially important not to allow your pollen "cans" to sit and bake in the hot sun while you work with your plants. By following these simple procedures, you can satisfy both requirements for maintaining pollen viability; 1) keeping the pollen at the lowest possible temperature and 2) preventing the pollen from losing moisture and drying-out to the point where it loses its fertility. The lesson here seems quite simple "dried-out pollen is dead pollen". However, keep in mind that pollen that is too wet (i. e., pollen that has

not been properly dried to begin with) will quickly turn moldy and spoil. So, in general, "wet pollen is also dead pollen". Therefore, it is important to find a happy medium here. The pollen must be kept dry enough to prevent mold, but yet moist enough to maintain its viability. This can be achieved by dehiscing the pollen from the anthers fairly quickly (1-2 days) and then transferring it immediately to a moisture-proof container for cold storage. (For more details on dehiscing pollen, see *Pæonia*, Vol. 26, No. 2, p. 2)

#### References:

Dumas, C. et al.,  $^1\text{H}$  Nuclear magnetic resonance to correlate water content and pollen viability, *Pollen: Biology and implications for plant breeding*, Elsevier Sci. Publ., Mulcahy and Ottaviano, Ed. (1983).

Wolfe, H. E., *The Peonies*, American Horticultural Society, J. Wister, Ed. (1962).

Washington) which produced 19 firm seeds. As of the end of Jan. 97, 8 (42%) of these seeds have germinated, although a couple of these seem somewhat reluctant to grow a good root. The rest, however, are safely in the refrigerator until spring. At this point, I am quite confident that these are true intersectional hybrids, but the possibility of contamination always exists. Undoubtedly, I will know a good deal more in a few months when these seeds begin to grow.

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### LESSONS IN GENETICS: PART I

#### *UNREDUCED GAMETES IN INTERSPECIFIC HYBRIDS AND THEIR IMPLICATIONS FOR THE ORIGIN OF NEW POLYPLOID SPECIES*

by Don Smith

#### *HIGHLIGHTS OF THE 1996 SEASON*

by Don Smith

In 1996 I continued to pursue various intersectional crosses and back-crosses in both directions. Overall, I obtained significantly fewer seeds than in previous years due primarily to the fact that I made considerably fewer crosses using A-199 pollen. I opted instead to experiment with pollen from a number of advanced generation Daphnis shrub peony hybrids with genetic make-ups that are  $3/4$  *suffruticosa* and  $1/4$  *lutea*. Pollen from Nike, Zephyrus and D-67 (F<sub>2</sub>B x Choni) used on Martha Washington gave a total of 26 seeds (2, 12, and 12 respectively), of which 6 (23%) have germinated so far. It is noteworthy that an unusually high percentage of these seeds (50%) were ruptured. The 96 season also brought my first success with an intersectional back-cross. A cross of [Miss America x "I" hybrid (mixed)] produced two very large seeds both of which have germinated. We will have to wait and see if these two are real intersectional hybrids. Without question my most exciting success, however, was from the reciprocal intersectional cross (*p. suffruticosa* x *p. lactiflora* var. M.

Hybridizers in many genera have recognized the importance of unreduced gametes and have successfully used this relatively common phenomenon to increase the ploidy level of hybrid progeny. In fact, it has been suggested that breeding tetraploids with the aid of unreduced gametes is much easier and cleaner than conversion of diploids to the tetraploid level by treatment with colchicine or other chemicals. Unreduced gametes probably occur in most genera, but are quite common in some genus such as *Lilium* where they are being widely used by breeders of polyploid lilies. The importance of unreduced gametes has yet to be fully recognized among peony hybridizers but this has not slowed the inevitable progress towards the development of tetraploid peonies from diploid species. Most individuals that produce unreduced (2n) gametes do so at a very low rate. However, exceptional individuals that routinely produce high percentages of unreduced gametes have been discovered. These plants, though rare, are of enormous value to breeders since they provide an important tool for producing polyploid progeny.

In general, unreduced gametes occur most frequently in the F<sub>1</sub> progeny of interspecies crosses between diploid parents. In many cases these F<sub>1</sub> hybrids can only reproduce by

unreduced gametes and thus the F2's and subsequent generation hybrids (F3's, etc.) are often fertile tetraploids. Sparkling Windflower, Nosegay, Gwenda and Daystar are all examples of tetraploid advanced generation hybrids that originated from diploid species. Due to the presence of unreduced gametes, it is inevitable that the descendants of interspecies hybrids from diploid parents will reach the tetraploid level. This was pointed out by Chris Laning in his article "Diploid Crosses" (Paeonia, Vol. 12, No. 2, p. 7) in June 1981, where he stated that the transition to the tetraploid level is usually accomplished by the F2 generation, and is nearly always completed by the F3 generation. It does not really matter whether the F1 hybrids are diploids or triploids, their descendants will just as surely reach the tetraploid level. Of course, the same can be said for the descendants of crosses where only one species parent is a diploid (and the other is a tetraploid), but this seems much less surprising. For the most part, this inevitable transition to the tetraploid level has already occurred with the herbaceous interspecies hybrid peonies and it is also well underway in the shrub peony hybrids. In time this process will occur with the intersectional hybrids as well.

Understanding the rules that apply and knowing generally what to expect from a particular cross can often help us make better choices concerning the use of our available breeding stock. Although, a certain result may be inevitable, the time that it takes to achieve that result can be greatly influenced by the approach that is taken. With this in mind, I have tried to summarize the "rules of the game" that apply when crossing diploid species or working with hybrids derived from diploid species. These "rules" are listed below:

#### Hybridizing with diploid species

1. As a rule, interspecific hybrids suffer a loss in reproductive capacity. In fact, the most characteristic feature of interspecific hybrids is sterility to a greater or lesser degree.
2. The formation of unreduced gametes appears to be particularly frequent in wide interspecies crosses between diploid parents, apparently as a result of the disturbances in meiosis that are associated with the lack of chromosome pairing. More important is the fact that

- Many such crosses are only possible when unreduced gametes are involved

And as a consequence,

- The F1 hybrids from such crosses between two diploid parents are often sterile triploids.

3. The F1 progeny resulting from these interspecies crosses (whether diploids or triploids) frequently produce unreduced gametes. In many cases, such hybrids can only produce viable offspring when unreduced gametes are involved.

4. Male gametogenesis is more easily upset by chromosomal or genetic disharmonies than female gametogenesis. Therefore

- Unreduced eggs occur more frequently than unreduced pollen

And consequently,

- It is usually more advantageous to use F1 interspecies hybrids as seed parents in backcrosses with species parents.

5. Many of the F2's and most of the higher generation progeny (including back-cross progeny) will be tetraploids irrespective of the ploidy level of the first generation hybrids.

Unreduced gametes are not only important in the development of new polyploid hybrids but also play a significant role in the origin of new polyploid species as well. One such example is discussed briefly below.

#### The Origin of a New Polyploid Species

##### An example:

When two diploid species of the annual Old World herb *galeopsis* were crossed as shown below

*Galeopsis pubescens* (2n=16) x *Galeopsis speciosa* (2n=16).

the result was a number of highly sterile F1 diploid plants (2n=16). Of six F2 plants produced, five were also diploids but one was a triploid (3n=24). The triploid was the result of the fusion of an unreduced egg with a normal pollen grain such that

$$2n_u \times 2n = 3n,$$

where the u subscript indicates the presence of unreduced gametes. When this triploid F2 hybrid was backcrossed to one of its' parents, *G. pubescens*, in the cross

***G. pubescens-speciosa* x *G. pubescens*,**

it produced a single fertile tetraploid (4n=32). Again, this was determined to be the result of unreduced gametes occurring on the female side, such that

$$3n_u \times 2n = 4n,$$

or, in other words, three sets of chromosomes from the triploid mother (one unreduced gamete) joining with a single, normal haploid set of chromosomes from the diploid father, such that  $3n + 1n = 4n$ .

Interestingly, this tetraploid hybrid was found to be morphologically and genetically indistinguishable from *Galeopsis Tetrahit*, a naturally occurring tetraploid species, except that it was clearly produced experimentally. This plant was self-fertile and crossed readily with *G. Tetrahit*. Therefore, it appears likely that

***G. pubescens-speciosa* x *G. pubescens* = *G. Tetrahit***

and thus the probable origin of one tetraploid "species" has been uncovered.

Undoubtedly, many naturally occurring tetraploid "species" have been produced in a similar fashion from diploid species. In those cases where the new tetraploid is self-fertile (such as in the example above), only five or six generations of in-breeding are necessary to

produce a population of true-breeding, self-fertile tetraploids, and thus a new species.

In addition, polyploids occasionally arise spontaneously within a diploid species. Both tetraploids and triploids have been found among the progeny of diploid species. The tetraploids are presumably from the union of two unreduced gametes whereas the triploids are probably the result of the fusion of an unreduced egg with a normal pollen grain. When a diploid population gives rise to a tetraploid population, the tet form may eventually supplant the diploid form; or both populations may continue to develop and diverge into two separate species with their common origin becoming obscured.

A few polyploid species are believed to have originated as a result of somatic doubling. However, evidence of this method of origin of polyploids is difficult to obtain among wild forms. Polyploid forms of *Solanum* were obtained as adventitious shoots following decapitation of a rapidly growing young plant. About 6% of the adventitious shoots formed during the rapid regeneration of the cut surface were found to have double the number of chromosomes of the original diploid plant. Twin seedlings may be another way that polyploids are created from diploid species. For example, it has been found that a significant proportion of the twin seedlings of the diploid *Gramineæ* have one member of the twin that is triploid; and haploids have been found among the twin seedlings of cotton, flax and *Datura*. One excellent example of the origin of a tetraploid species by doubling of the chromosome number in the somatic tissue is that of the tetraploid *Primula Kewensis*. The sterile diploid hybrid from the cross between *P. floribunda* (2n=18) x *P. verticillata* (2n=18) produced one branch with fertile flowers. This branch was found to have double the chromosome number of the rest of the plant tissue. Offspring obtained from this branch were fertile tetraploids (4n=36) which bred approximately true and became known as *P. Kewensis*. Overall however, many more polyploids originate by irregularities in meiosis in the parents (i.e., from unreduced gametes) than from doubling of the chromosomes in the somatic tissue.

#### References:

Sansome, F. W. and J. Philp, Recent Advances in Plant Genetics, P. Blakiston's Son (1939).

## LETTERS TO THE EDITOR OF PÆONIA:

The following article on Fan Tan was received from Harold Entsminger of Cut Bank, Montana in response to my article "Unsolved Mysteries of the Orient..." in the last issue of the newsletter (Vol. 26, No. 4).

### QUALITIES OF FAN-TAN

by Harold Entsminger

A recent acquisition of *Fan Tan* gives me some ideas on one of Don Smith's questions in the Fall 1996 issue of *Pæonia*. Can *Fan Tan* be an intersectional hybrid?

Within my new raised planting of over 100 varieties of tree and herbaceous peonies (85% herbaceous, 15% tree), *Fan Tan* was the first variety to emerge or show signs of life in the spring of 1996. Its rhubarb red stems covered in white fluffy stuff pushed skyward. Fungus? No! Indumentum and tomentum matted on the stems and on the leaf's under and upper surfaces, more indumentum and tomentum than I've have ever seen on any peony. Shock waves went through my head upon seeing this sight for the first time. Then, visions of Yakushimana's (island of Japan) high, moist mountain areas focused in my mind. Could *Fan Tan* grow side by side with *Rhododendron yakushimanum* in the frozen mountainous areas? It's possible. What implications! Possible Species? Hardy, tall, vigorous, *P. Fan Tan* is floriferous. On hardiness..... We had many nights of -30° F (and one of -56° F) during the spring of '96 here in Montana. On fertility..... Chris Laning told me "I've tried crossing *Fan Tan* with every herbaceous variety I can think of and nothing! It just won't set seed. I was so excited about the hybridizing possibilities at first, but now, years later, I give up. I don't see anything fantastic about *Fan Tan*." Now there's an intersectional quality for sure. Should sufficient pollen present itself, I would like to try the cross *p. mloko. x p. Fan Tan* and also *p. Tessera x p. Fan Tan*. *Tessera*, D-4, has no pollen, therefore, cross contamination chances are minimal and it does set large viable seed, usually only one or two per pod. I already have one healthy seedling growing from *Tessera x Prairie Moon*, so I am eager to see what I'll get. So I will continue Chris' work a

little longer and see what's shakin'. Hey, its fun! Chris Laning has roots of *Fan Tan* for sale if anyone is interested in applying their own genetic thoughts.

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**Editorial Note:** More on the *Tessera x Prairie Moon* intersectional seedling mentioned above will appear in the next issue (Spring 97) of the newsletter.

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The following letter was received from Chris Laning

Dear Donald:

About 20 years ago I bought a plant of *Fan Tan* from Smirnow, hoping to find it useful in my quest of a fertile yellow herbaceous clone. For many years I had worked with it without success. Finally patience was exhausted and I turned to other clones offering at least a promise of some measure of success. Now I have a short row of these *Fan Tans* (always hoping to find an occasional viable seed) but finding the whole deal a poor investment.

Because *Fan Tan* may not be readily available, I'm sending you a whole plant of it. Now, you work with it!!

You ask: "Where could these uniquely lutea flower colorations come from?" The answer is from the mind and the imagination of the Japanese. In Smirnow's first offering he was careful to state that *Fan Tan* flower color was the Japanese description (a coy attempt to disassociate himself from that description). That was a wise move on his part!

Keep up the good work!

Sincerely,

Chris Laning