PAEONIA

Volume 13, No. 1 March, 1982

REQUIRED READING:

1. "The Peonies" by John C. Wister, $3.50 from American Peony Society, 250 Interlachen Rd., Hopkins, MN 55343
2. Bulletins of the American Peony Society.
3. History of the Peonies and their Originations.
4. The Best of 75 Years; 3 & 4 ed. by Greta Kessenich, and available from the American Peony Society.

Editors are Chris and Lois Laning,
553 West F Avenue,
Kalamazoo, Michigan, 49007.
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FINAL OFFER OF SEEDS (Plant in June!!)

(From Don Hollingsworth)
1. Mixed earlies #1 - from cream and yellow parentage - a few others mixed in.
2. **Rushlight** F_3 or F_2
3. Krekler's Seedset - officinalis type (open pollinated).
4. Mixed earlies #2 (**Roselette's Child** F_2 - **Roselette**'s Child F_2 x Moonrise, etc.
5. Roy Pehrson's **Silver Dawn** F_3 seedlings - early herbaceous.

(From Gus Sindt)
1. '**Seashell**' -pink single
2. '**Krinkled White**' (single)
3. '**Pico**'
4. '**Rose Bowl**' - pink single
5. '**Dawn Pink**'

(From Domoto - and a few from Laning)
Tree Peony - (suffruticosa)

* *

Since the last issue of PAEONIA was sent to you, we have received notice of the death of three of our hybridizers —

Helen Titus, Derby, Kansas
John Van Zandt, Washington, Pennsylvania
Roy Pehrson, Lafayette, Minnesota

The Passing of Roy Pehrson leaves a vacant place in the happy family of hybridizers. Poor health stopped him short of realizing his goals. Recognizing this condition, he willingly distributed his valuable hybridizing materials, greatly benefiting others. His teachings and instructions will be sorely missed, as will Roy Pehrson! – Chris

Reprint from PAEONIA - Vol. 10, No.1, March 1979

SYNTHETIC TETRAPLOIDS
(Syn-tets)

Roy Pehrson

I don't hesitate to declare that at some time in the future these plants and their derivatives will be used, almost to the exclusion of everything else, in peony breeding. Someone of more scholarly bent will, hopefully, use this information to write a more definitive treatment for publication in the Bulletin. In this account I will give only a "bare bones" step-by-step outline of how it works.

Step No. 1
Gather pollen from a tetraploid species, or from any other tetraploid in the genesis of which the species lactiflora has played no part. Use this pollen on seed-bearing lactis. If seeds are obtained, the resulting seedlings will be very sterile triploids.
EXAMPLES: The Saunders' lobata hybrids, the Quads, etc.

Step No. 2
Such a sterile triploid may very rarely produce a well formed self-set seed. When grown on it may very well produce a fertile tetraploid of great breeding promise. For the purpose of this account I have dubbed these "syn-tets" because of that unorthodox origination as F_2 plants from triploid parents. They also behave in an odd manner when an attempt is made to backcross them to lactis.
EXAMPLES: '**Moonrise**', Quad F_2, '**Archangel**', '**Sanctus**', etc.

SOME OBSERVATIONS
Thus far, whenever an attempt has been made to backcross one of these original F_2's to lactiflora, the result has always been the same. Large, apparently well-filled seed pods are formed, but when these are opened at harvest time they are found to contain hollow seeds, with only an occasional good one among them. This trait does not disappear in succeeding generations, whether these result from selfing or from intercrossing. Even the use of pollen from a more conventional type of tetraploid, ('**Silver Dawn**' F_3) did not eliminate or dilute this trait. This peculiarity should be no obstacle. The influence of lactiflora's genes will remain constant from generation to generation in any event. Considered as a single strain, these syn-tets possess an easy growing habit which has never before been realized in any tetraploid strain or in the diploids either. Hybridizers should pay them their fullest attention.

DON'T FORGET! MAKE THE CROSS — **MIKADO** x **GOOD CHEER**
IT'S THE CROSS OF THE CENTURY.

Take a look at the list of seeds offered for distribution in the September PAEONIA. Numbers 1, 2, 3, 4, 6, 7, 8, 9, 10, 13, 16, 17 and 19 are all "syn-tets". That's 13 of the 19 or 2/3 of the total. If 12, 13 and 14 are eliminated from consideration because they are not hybrids at all; the proportion rises to 13 of 16 — even more impressive. I think you will agree that this little exercise illustrates in a dramatic way how hybridizers are being led, unintentionally, to the conversion of a large segment of the herbaceous peony list to the tetraploid condition. This is really a remarkable development, even though it has been completely unplanned.

HYBRID PEONIES AND CHROMOSOME BEHAVIOR

Don Hollingsworth

Questions: Why are there no syn-tets (man-made tetraploid strains — "synthetic") in the tree peonies — suffruticosas or lutea hybrids. Why isn't Laning's Best Yellow pollen effective on lactiflora peonies?

I can only comment on these questions. My estimate is that the answers are something like a course — nay, a major study — in genetics. I do have some thoughts to share and can suggest some references.

I believe the problems raised involve the genetic makeup of the plant and groups of plants mentioned. Genetic "sterility" factors may be involved — they've been postulated in Paeonia by some imminent authorities — but there is an overriding problem of the nature of chromosome behavior during meiosis that is common to all organisms which reproduce sexually. These subjects have been studied by geneticists — decades ago — and some of the study was done on peonies and on the Saunders collection of hybrid peonies in particular. Therefore, it will be rewarding for peony breeders have more than a casual interest to look up some of the references.

The significance of the above questions for breeders is more "how" than "why." How to overcome the apparent barriers to fertility that are contained in the hybrids. Maybe it will help to examine some of the differences in fertility between hybrids at the diploid vs. the tetraploid levels. Notice I said hybrids. Colchicine-induced tetraploids from non-hybrid origin are likely to have fertility problems, but I don't want to introduce that into the discussion this time.

Meiosis is the reduction division which takes place during formation of the germ cells of pollen and ovary. The pairs of chromosomes divide so that when meiosis goes forward ideally, one member of each pair goes to each daughter cell so that each germ cell has a precise half set of chromosomes.

In order for the reduction division to take place most accurately and reliably, the ideal is that the two members of each pair of chromosomes be exactly alike, bit by bit, along the full length of the strands. During the pairing process like attracts like. The strands which find like parts in other than the ideal location are subject to many aberrations of pairing, including attraction to other chromosomes, formation of loops, etc. For these and other reasons, breaks occur at separation, sometimes to rejoin at different places or even parts lost. This is just an illustration of the sorts of consequences of imperfect matches between homologous chromosomes. A lot of it can be tolerated, so long as at least a fair amount of good germ cells are formed, and there will be fertility. In nature, the species tend to develop mechanisms which drastically reduce the development of these difficulties in pairing and the consequent loss of fertility. Lineages which fail to maintain fertility simply become extinct.

When we set out to break down the species barriers, we also find some ways that new species may arise in nature. We seek variability and we keep messing with infertile hybrids to overcome their infertility. One of the ways this happens is the emergence of tetraploidy. The greater the variability between the parent sets of chromosomes contained in a hybrid individual, the more problems that arise and the fewer chances of the formation of good germ cells. Sometimes there will be either a failure of pairing or there may be a configuration where some part of every chromosome is attached to two others so that all the chromosomes are hooked together. These situations may lead to no cell division rather than divisions having wrong numbers in the daughter cells. With no division, a diploid set of chromosomes goes to the germ cells. This is a viable germ cell having a balanced set of genetic material. If it is joined with another diploid germ cell at fertilization, a tetraploid is formed. This tetraploid may be fertile, either some fertility or highly so, depending again on how good are the pairing partners available. If the new individual now represents very divergent species, we may have somewhat of the same problem as we had at the diploid level as far as pairing is concerned. However, if the distribution of chromosomes is such that all or most of them have a potential partner for pairing that is more alike than the two are like any other in the group, pairing can work out effectively a high proportion of the time and fertility is likely to be satisfactory.

The effect of imperfect pairing does not lead to a yes-no dichotomy of fertility. Rather there is a continuum from little to very high potential of fertility. In addition to the gradations of good germ cell production, successful breeding depends on the production of sets of chromosomes that together with the mating partner's set of chromosomes adds up to a balanced set of genetic material sufficient to support the development of a mature offspring. Thus, producing good pollen and good egg cells, only gets us part way there. If the seeds are faulty or the plants weak, they further reduce our results.

The bottom line is not only can we produce seeds, but can we turn some of them into vigorous plants? We always look to the point in time when we can grow large numbers of seedling from a desirable strain, so that the best chance of finding the best possible selection from the strain is assured. However, any level of production that permits progress from high infertility toward fertile types, which also contain the characters we're trying to get, constitutes a gain. Thus, to say Laning's Best Yellow is not effective on lactiflora peonies requires that a subjective standard of effectiveness is being set. I have a little experience crossing it on lactiflora and nothing has happened so far to discourage from trying some more. As I have observed the results, LBY is not one of those tetraploids that are giving a high proportion of well-balanced sets of genetic material. Some others are '**Archangel**' and '**Moonrise**'. However, we are getting some seedlings and one of these may be worth more than a thousand from some single flowered tetraploid that is otherwise similar. I think it is more useful to ask the questions, "Do I want some progeny from this plant?" and "How diligently am I willing to work and spend resources to get them?"

When hybrids have been formed from two diploid species, such as the lutea hybrids, the like chromosome between the two parent sets will carry substantial differences, in all but the closely related species. This can be expected to result in relatively great sterility. If, however, a plant can be produced from one of these in which the original number of chromosomes has been doubled, we now have a tetraploid in which each chromosome has an ideal partner for efficient pairing.

I have no clues from research references as to why there are no tetraploids in the suffruticosas. There is a tissue culture study in which both tetraploid and octaploid cell lineages appeared. This study involved culturing callus tissue and changing it to new medium periodically (sub-culturing). One of the things that happened was that the proportion of polyploid cells would rise and fall, as though the lineages were playing out, unable to maintain sustained growth. Perhaps the rise would be due to appearance of new mutants (Demoise, doctoral dissertation. U. of Pittsburgh, 1967).

Stebbins speculated that there is a prevalence of genetic sterility factors operating in peony species that help to keep the species isolated from freely crossing in nature. This was 40 years ago. I have no idea whether he has advanced these thoughts at all in the meantime. The Stebbins references on peony studies are listed in my article in APS Bulletin 236, Dec. 1980, except for one. This is Hicks, G.C. and G. L. Stebbins, Jr. "Meiosis in some species and a hybrid of Paeonia." American Journal of Botany, 1934, vol. 31, pp. 228-241.

These papers can be seen and copied at science reference libraries such as are maintained at universities. I can also assist in obtaining copy services.

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<u>AMPHIDIPLOIDS</u>

THIS I BELIEVE: What Roy Pehrson identified as Synthetic Tetraploids (syn-tets), I think of as amphidiploids. This conclusion has been reached by observing results of hybrid plants being used in my breeding program.

"AMPHIDIPLOID = a hybrid between two species that has at least one complete diploid set of chromosomes derived from each ancestral species." Page 433 - Glossary - in "The World of Irises" by Warburton and Hamblen. In the same book, on page 390 on this subject this paragraph is apropos! "The most regular of the allotetraploids with nonhomologous genomes are the amphidiploids with two chromosome sets from one species and two chromosome sets from a different species. As the name indicates, such a plant behaves as a diploid for both chromosome sets. At meiosis the two chromosome sets from one diploid species pair with each other as do the chromosome sets from the other species: each chromosome has one and only one homolog and pairs only with that one. Each gamete therefore contains one complete set of chromosomes from each species. Such tetraploids generally are fertile because meiosis is regular."

Sorry about all these big words but without them a clear concept of this theory (or rather - this fact) cannot be conveyed.

But what does it mean? Well, don't backcross hybrids onto lactiflora clones!! The very limited seed production and the amount of seedlings thus produced will be very small. Also these seedlings will be infertile (highly sterile).

I believe almost all our hybrids in the herbaceous group are amphidiploids.

Also, I believe that there are no syn-tets, - no amphidiploids? - no tetraploids in the suffruticosa (tree peony) species or its hybrids. This includes the lutea hybrids as well.

- Chris Laning

Dear Chris, January 4, 1982

It's January again. Time to send my Paeonia dues for 1982 and more particularly, to express my appreciation for the work you undertake and the responsibility you assume in putting out the newsletter. It contains the type of information hybridizers need and cannot get in the A.P.S. Bulletin.

From the Laning's Best Yellow pollen you sent, I now have the following seeds in the early germination process.

'**Laura Magnuson**' x L.B.Y, - 1 seed;
'**Paula Fay**' x L.B.Y. - 1 seed;
'**Alexander Woollcott**' x L.B.Y. - 2 seeds; and
'**Old Faithful**' x L.B.Y. - 45 seeds.

From past experience I would guess that at least 1/3 of the above will develop into viable seedlings. I do hope that my successes, if any, are concentrated in the first three mentioned crosses.

From a color standpoint I can't imagine what the result of the '**Old Faithful**' x L.B.Y. cross will be. However, if the offspring take after the seed parent as far as stems are concerned, they will have more than adequate stem strength. I have never seen a peony with stems like '**Old Faithful**'. I believe I have read in the Bulletin that it is a fourth generation Glasscock seedling, introduced by Reath. And it obviously is a good seed setter.

Unlike last year we have an ample snow cover and so hopefully can avoid the winter kill that we all had last year in this area.

Yours truly,

Dick Edblom
6917 4-5th Ave. N.
Minneapolis, MN 55428

Box 27
St. Norbert, MB R3V 1L5
Canada

Dear Mr. and Mrs. Laning, December 15, 1981

I have just been rereading some copies of Paeonia, and thought I would write to express my appreciation and encouragement to you in your efforts with the newsletter. I don't know how my account stands just now, but I enclose $5.00 to keep Paeonia coming. I'm sorry I can't contribute to Paeonia in any other way. I'm still a beginner. I have a dozen or so small plants coming along from seeds you sent me in previous years. I would have more if I had realized how I should protect them from burrowing animals and frost heaving, but I'm learning.

Over the past several years I have assembled a good collection of peony varieties, including many hybrids. These suffered very severely from the Red River flood in May, 1979. The plants were just emerging from the ground when they were covered by the flood water. They were under about a foot of water for two weeks and in saturated soil for another two weeks as rains followed the flood. All above-ground growth was killed but only about a dozen plants out of about 100 were completely killed. Small new shoots continued to appear through most of the summers and a few didn't show life .until the following spring. But all were very set back, often reduced to a few small fragments around a rotting core. Plants put in the preceding fall were most sensitive, usually being killed while more established plants usually lived at least in part.

I now have all my peonies moved to higher, better drained ground, out of flood danger. There are lots of plants — small but growing. A few bloomed this year, and I look forward to more next year, and even some crossing in a few years' time!

Best wishes to you both!

Sincerely,

F. P. Healey

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Item from - "Genetics of Flowering Plants" by Verne Grant

Page 394:

"It has not been possible as yet to grow these (Paeonia californica) peonies from seed to maturity in the experimental plot."

This is another aspect of peony population biology warranting further study.

MATERNAL INHERITANCE IN PLANTS

Don Hollingsworth

It has been stated that if two plants are crossed one way and then the other (the reciprocal cross) the progeny may vary upon the basis of which is the seed parent. (For example, Wister, John C., ed., The Peonies, 1962, pp 43-44.) What makes this possible?

Professor A. P. Saunders is credited with this conclusion in respect to peonies, but the idea has been a part of the beliefs of animal and plant breeders of other species. Geneticists have determined some ways in which this may be happening.

The problem of lack of awareness about this subject is probably related to the fact that the teaching of Mendelian inheritance centers around the study of what can happen during the divisions of the nucleus germ cell formation. Concepts such as genes, alleles, segregation, independent assortment, linkage, crossing over, mutation and recombination have all arisen out of study of the nucleus. This has generated a strong expectation that the range of progeny characters should be the same irrespective of which parent contributes the ovum and which contributes the pollen germ cell. Since the same forces govern the reduction division (meiosis) wherever it takes place, a particular combination of genetic material should have the same opportunity of being produced either in the ovary or in the anthers of a given plant. These remain the appropriate expectations insofar as the nucleus is concerned. Indications are that maternal inheritance in plants operates outside of the nucleus.

The germ cell of a pollen grain is made up predominantly of the nucleus which it carries. The egg cell or ovum is, in contrast, very bulky. Thus, the bulk of the zygote (fertilized egg cell) reflects principally the original bulk of the egg cell. How might this non-nuclear content come to bear upon the character of the progeny?

It is not the relative bulk of the two contributions that must be looked to, but the question, "What are the possible heredity determinants that could be involved?" What is not so widely understood about genetics of plants is that DNA strands occur outside the nucleus, contained in certain organelles of the cell (an organelle is to a cell as an organ is to a whole organism, a functional sub-unit). One such DNA-containing organelle is the plastids (chloroplasts). Another is the mitochondrion. Both of these have double stranded DNA. Another organelle, the ribosome, has RNA that resembles the RNA of bacteria.

Chloroplasts contain genetic information sufficient to lead to the manufacture of several hundred proteins in certain algae. These would be enzymes and capable of influencing development in some way. In higher plants chloroplasts increase by simple division, which is not correlated with division of the nucleus. However, the extent to which these divisions go forward independently of the influence of the nucleus is unknown. Chloroplasts are not seen as such in embryonic tissues, but they arise in the cells from proplastids. Pollen cells contribute fewer proplastids to the zygotes than do the egg cells. (The foregoing was extracted from Rothwell, N.W., Understanding Genetics, 1976, Williams and Wilkins, pub., and Jinks, J.L., Extrachromosomal Inheritance, 1964, Prentice-Hall.)

This is just a sampling of the literature of maternal inheritance in plants, taken from general references that are likely not up to date on the subject. However, it probably suffices for the purposes of planning crosses. What difference does it make in peony breeding?

My view is, it reminds us that when we can arrange to do so, we ought to make our crosses both ways. However, this is going to be limited to those instances when we have useable levels of fertility on both sides of a cross and when we can have both parents in flower at the same time (or acquire pollen in some other way).

In many of the crosses in which I am still interested the fertility of one parent is so low that I can seldom get a seed from it, but can do fairly well when using its pollen on a fertile seeder. In this situation, the chance of finding wanted differences would be severely handicapped by the difficulty in obtaining progeny. However, once the possibility of obtaining more desirable plants from a reciprocal cross is established, one would have no reluctance to pursue a cross just because it is difficult. The key will be how to interpret observed variations in results.

The numbers of progeny necessary to rule out Mendelian segregation of genes as the cause of variation becomes staggering as the number of gene locations which influence the result increase, and as we go from diploid to tetraploid. The present hybrid-origin tetraploid strains represent an enormous potential for variation through segregation of nuclear genes alone. If we set about trying to find maternal inheritance it will be easy to think up some for no other reason than we have a relative large number of variants and a modest number of progeny from either the cross or the reciprocal.

The mathematical chance of getting an individual that is homozygous recessive in each of a three-locus complex from a cross of tetraploid parents that are "2-2" hybrids (as BBbb) at each locus is one in 46,656. But, that chance exists for each individual embryo formed. Suppose a desirable one of them shows up in a reciprocal cross which produces few plants and it shows up early on? Isn't there a very good chance that any of us who is aware of maternal inheritance is going to look to that for the answer? If we're wrong we make a mistake if we abandon the other, perhaps more fertile direction, of the cross. If we're right, however, it should pay off to keep repeating attempts to make the cross in the difficult direction.

So, while the mechanisms of maternal inheritance offer an interesting subject for study, it is not now the sort of phenomenon around which one could build a breeding program. At the same time it may turn out to be the preferred explanation for unexpected results somewhere along the way.

JUDGING TETRAPLOIDY WITHOUT COUNTING CHROMOSOMES

Don Hollingsworth

Question: Are there some morphological characteristics of the progeny which you use to identify tetraploids? If there are such characteristics, I could be looking for them in my colchicine treated seedlings.
L. J. Dewey

Answer: In the first place, it is David Reath that has the direct experience with induced tetraploidy in peonies and the selection of progeny for further tests — specifically, chromosome counts. I hope this question will receive comment from David.

There is a method used in evaluating dried specimens in herbarium collections to infer tetraploidy, when both diploid and tetraploid specimens are available. G. L. Stebbins, Jr., a biosystematist who worked extensively in the study of the Saunders collection and published a significant alternative to F. C. Stern's synthesis of species relationships in peonies, described his use of this approach:

> "Since in some of the species complexes, . . . knowledge of the chromosome number is essential to an understanding of their systemic relationships, a method of inferring this in herbarium material has been of some value. This was measurement of the length of the guard cells of the stomata, a method used also. . . (by others). Since the length of the guard cells varies with the position of the leaf on the stem, being shorter in the higher leaves, care was taken to obtain the samples for measurement from corresponding parts of corresponding leaves. In this study the side of the terminal segment (leaflet) of the middle cauline leaf was selected. (Meaning the fully formed leaves of the stem, not those smaller ones below the flower.) Although variation in size was found, the average size for the known diploids was always significantly lower than that for the tetraploids, the former ranging from 41 to 45 microns, and the latter from 47 to 52 microns. It was slightly larger in fruiting than in flowering specimens, so that when the maturity of the specimen was taken into account, an even greater contrast between the two types was found than is indicated by these figures." page 247, Stebbins, G. L., Jr. Notes on some systematic relationships in the genus Paeonia. Univ, of Calif. Publ. in Botany, Vol. 19, No. 7, pp. 245 - 266.

If one has access to a microscope and a scale for making the measurements, this approach should help. Perhaps a little review in a botany text on leaf anatomy would be useful.