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Variations and Peculiarities in Plant Breeding

H. William Barnes

Barnes Horticultural Services, Warrington, PA

bill@barnhortservices.com

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INTRODUCTION

Plant breeding can be approached via a variety of avenues. Time tested methods of hand pollination either by direct transfer by fingers, popsicle sticks or artists brushes are still quite valid and useful. Some new techniques include caging plants with bees, and using mechanical devices such as electric tooth brushes to dislodge pollen. Still other methods can be as simple as interplanting two different cultivars or plants in a given area and let natural forces do the job.

Simple crosses such as corn hybrids can be done by planting two types adjacent to one another and emasculating one clone so that it can only be pollinated by its neighbor. Similar techniques are used for the crossing of *Castanea dentata* hybrids. But these applications work only when standard Mendelian Genetics apply. In the case of annuals specific clones are back bred to F6 status. This eliminates most if not all extraneous genes so that the crossing of two F6 clones result in a specific given hybrid. It could be safely assumed that a cross of a specific red petunia with a specific white petunia will give rise to a chosen pink petunia. However, when it comes to woody plants and some perennials it is not always possible to go to the F6 status. As a result, the offspring of crossing two distinct but variable individuals will result in significant variation in the resulting seedlings. Of course, this in turn requires the rigors of planting out and selection both visually and environmentally to achieve a desired goal. Somewhere, somehow, the bulk of the offspring under testing have to be dispatched and eliminated in preference to the best possible choices.

Many of today's roses are the product of such crosses where for instance Mr. Lincoln is crossed with Chrysler Imperial. The results of such a cross will be quite varied and will at times give some unexpected results. In work at Barnes Horticultural Services a cross of *Hibiscus syriacus* hybrid 'Blue Bird' as the female parent and *Hibiscus* x*tosca* (*H. syriacus* x *H. paramutabilis*) as a male, gave rise to 15 different individuals based on form and flower color. Variations include those that looked like the parents but also a vivid pink, a dwarf white and a fastigiated purple amongst the 15 individuals. Some are worth keeping and others not so. Some had unique leaves but non-descript flowers. Again, selection pressure and evaluation were applied to discover those with the best merits. It should be noted that this is with plants that are chromosomally compatible. But when disjunct and poorly coordinated chromosome pairings are made problems immediately jump to the forefront.

In *Capsicum*, there are two separate lines based upon chromosome numbers (12,14), one line is 2x= 24 and the other is 2x=26. Crosses between these two lines is not possible and stymies attempts at breeding for acquiring novel traits. However, if these lines are doubled to tetraploid then crosses could be made but the resulting offspring would be most likely sterile. Researchers (personal communications) at the University of Florida have told me that they cannot get large fruited hot peppers to cross with small fruited hot peppers even if the chromosome numbers are compatible and they are mystified about why this situation exists. Possible answers to this which will be discussed further along in this paper.

In my personal work I have found that crossing *Hippeastrum x Clivia* will work but only in that direction. *Clivia x Hippeastrum* will not take. The late Luther Burbank (1914) suggested that reciprocal crosses will be just as effective as the opposite crosses but sad to say he was wrong.

Explanations as to why this might be the case are complex and varied. But sometimes things can be elucidated. In the Genus *Hibiscus* timing of pollination seems to be a consistent pattern. In Zone 6, *H. syriacus* stigma are not generally receptive until 10 AM and pollen generally is not available prior to 10 AM and sometimes matures much later than 10 AM. However, this is temperature related and things can speed up with warmer ambient temperatures, although it is not clear if the stigmatic surface receptivity functions on the same pace as pollen maturing. Cooler temperatures result in significantly delayed pollen maturity. These mechanisms seem to be a device to limit self-pollination and serve to foster outcrossing. It should be noted that many of the cultivars of *Hibiscus syriacus* are self-sterile and out crossing is a must. However, *H. syriacus* goes one step further and generally shuts down the whole operation at 2 PM. Few if any pollinations will occur in the *Hibiscus* after 2 pm. Like the morning phase the afternoon phase can be altered by temperatures being hotter or cooler on any given day. Things are speeded up with warm temps and delayed with cooler ambient air temps. I work with about 40 species of *Hibiscus*, *Abutilon* and *Pavonia* and they all follow similar timing regimens. This of course makes me wonder if other plants have similar built in "rules" on outcrossing. Perhaps this is the source of the reluctance of the *Capsicum* at the University of Florida not following a predictable pattern. It may be predictable, but it simply is not known what it is.

Several factors that govern pollination effectiveness include:

a. Pollen is not ripe at the time of pollination.
b. Stigma surface is not receptive due to poor timing.
c. Stigma surface is not receptive due to a hostility to incoming pollen.
d. Temperature and humidity are unsuitable for effective pollen germination.

Pollination involves two processes - pollen germination upon sticking to a conducive and receptive stigma followed by pollen tube growth allowing for a pathway for sperm to move down into the ovary. Another aspect of the Malvaceae, is that the pollen often varies with a certain degree of stickiness. Some pollen when fully mature will be extremely sticky and will adhere to just about anything, at other times the pollen will not adhere to brushes, or other implements or for that matter to insect pollinators. Unless conditions are exactly in sequence and in complete agreement an effective pollination will not occur.

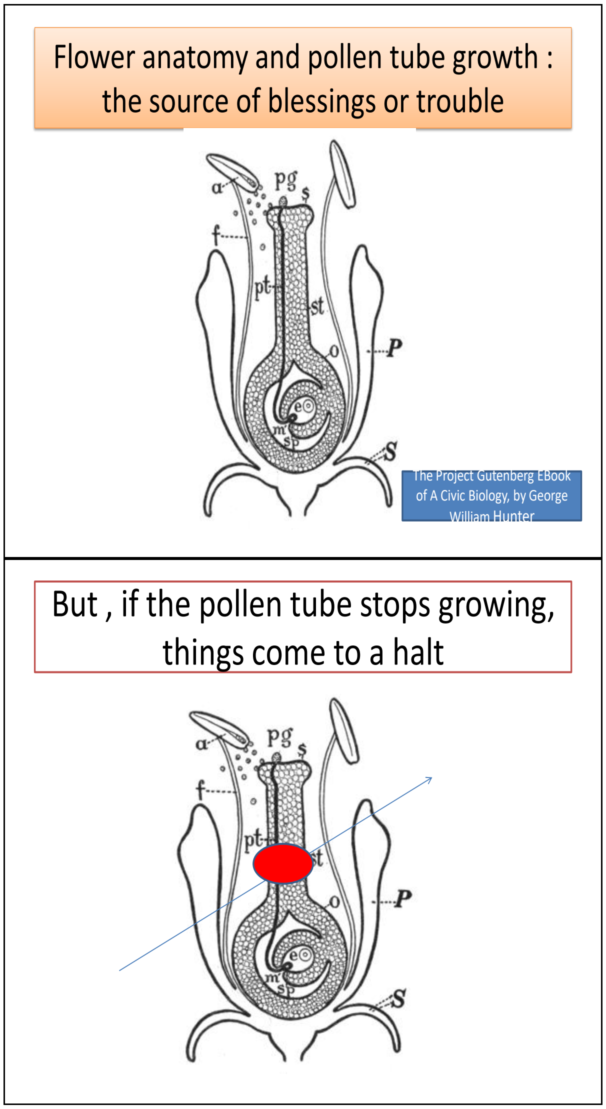

Figure 1. Pollen tube growth impacts successful fertilization.

The female/mother plant controls all of the interactions with pollen and has complete control over whether a successful pollination comes about. The process is not fully understood but somehow the mother plant can detect unsuitable pollen. Most likely this is chemical in nature, but the exact mechanisms are not known. Undoubtedly each species of pollen has unique signatures that the female plant can interpret with rejection appearing to be the norm, primarily to prevent inbreeding and self-pollination. This system also applies to pollen where the chromosome numbers are out of phase or if it is a foreign pollen that has no merit for the female. Think *Quercus* pollen landing on a *Hibiscus* stigma. The chemical barriers will prevent the pollen from germinating and if by chance it does then there are other agents in service to her majesty that come into play. However, this runs contrary to the role of a plant breeder who is trying to circumvent the processes and get two plants to form a union.

Hibiscus rosa-sinensis offers an interesting look into some of the nuances found in the plant world. Of the multitude of cultivars known most are sterile, of the fertile clones breeding research at BHS shows some 25% of them are yellow flowered. Having the fertility of yellow flowers being so high in comparison to the rest is a bit of an enigma. Do the genes regulating fertility have a link to yellow pigmentation? It should be noted that in the genus *Hibiscus*, many of the flowers especially of the species found in Africa are yellow. The highly colored forms of *H. rosa-sinensis* in comparison are out of phase with the genus as a whole. The late Barbara McClintock (Creighton and McClintock, 1931; McClintock, 1950) showed that plants do have linked genes and this situation is not an anomaly. But the key question is why is yellow the chosen color for fertility? This would be an interesting research project should someone somewhere have the time and interest to pursue it.

What about the "Controlling Hand"? Can we as plant breeders bring our expertise to bear on fooling the system and getting successful pollinations that otherwise would not occur. A number of chemicals are known to affect flowering, pollination and seed formation. (Addicot, 1943; Brewbaker and Kwack, 1963). These include auxins, gibberellins, cytokinin, ethylene as well as sugar.

This is not unexpected but what is somewhat of a mystery is how the relationships of the various hormones and chemicals play a role in pollination and seed formation. The question that comes to mind is, can we overcome barriers to pollination. The answer seems to be yes, but the systems are not necessarily straight forward. We can apply auxins, gibberellins, and cytokinin, we can use ethylene either as a gas or a liquid application with products such as Florel (Chacko et al., 1976; Costa, 2012; Rajasekharana and Ganeshan, 1994) We can alter both temperature and humidity to be optimal. As demonstrated with the *Hibiscus* genera we can alter receptiveness and the timing of pollination. These things are possible, but the application is more of a jigsaw puzzle than something more straight forward.

There are other chemicals when applied to flowers are known to influence pollination. Calcium salts, and boron compounds, are two definite agents (Brewbaker and Kwack, 1636). Other possibilities are manganese and zinc salts as well as napthyl acetamide (a synthetic auxin derivative) is well known to influence the process (Addicott, 1943). Sugars such as fructose and sucrose (glucose+fructose) seem to have an influence as well.

There are other tricks that can be applied to fool the system. One is to mix an acceptable pollen with that of a pollen that is not readily accepted. Another is to mix a killed acceptable pollen with that of a pollen that is not readily accepted (Jones, 1920).

One of the biggest dilemmas that breeders have is when two plants bloom out of phase. *Hibiscus* from Africa such as *H. platyphyllos* (yellow flowered) always wants to bloom in fall and winter in the Northern Hemisphere. Of course, potential Northern Hemisphere natives want to boom in spring and early summer. Trying to get the two trains to meet in the dark is challenging. Or consider the yellow flowers of *Cornus officinalis* and *Cornus mas* which bloom early in the spring but a good recipient such as *C. kousa* blooms several months later. There are examples of manipulation of environment to get two strangers to connect but it is tedious and not always reliable. However, pollen storage might be a useful key to solve the problem. There are problems. Many pollens do not store well and are short lived. Storage varies from species to species and they are not created equal. Liquid nitrogen storage of pollen is a given (Gaseshan, 1986; Pozzobon et al., 2006; Rajasekharana and Ganeshan, 1994). But it is hard to handle, it is expensive, and most operations are not readily equipped to deal with liquid nitrogen storage. Are there alternatives?

Using the techniques outlined by Jain et al. (1988), Kopp et al. (2002) and Parfitt and Ali (1983) indicate that a standard home or commercial freezer at -20C /-30C will do the trick.

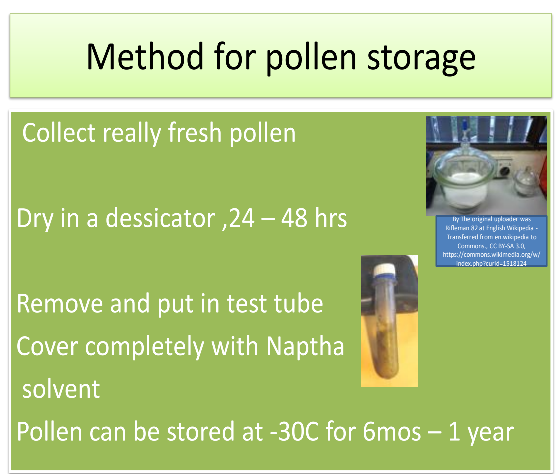

Figure 2. Methods for storing pollen.

Other Issues in Breeding

A common problem in breeding is that the fruit does form but the seed is hollow. Enough fertilization occurred to get the system going but then most likely the pollen tube failed, or the gametes were killed once they entered the ovary. A bigger problem is for seed to be normal and filled but fails to germinate. While embryo rescue will work it is restricted in application and out of reach of some breeders. A third issue is when normal seed germination can be offset, and a new regimen installed. For example, seed that normally would take one season to germinate will shift to a two-year cycle. Seed can also exhibit very poor germination, but something does get through the process. However, many of the resultant seedlings can be sickly and will die, leaving but a few examples of the hybridization. Those that remain could be well worth the wait by hanging on to for further evaluation.

Eureka, all systems are go and the result is a bonafide hybrid seedling. But and this is a big but, the resultant plant will not bloom. Sometimes nothing applied, altered or manipulated will change this and the plant simply will not bloom. This situation can occur especially when a wide cross is accomplished by manipulation. Chances are there is such misalignment of genes and chromosomes that some systems are permanently turned off. Grafting onto a more freely flowering root stock might be a help and this is a technique that Luther Burbank employed to great use. (Burbank, 1914) But it is not always a cure all.

Can we be sure a cross pollination has been successful? Sophisticated labs make use of flow cytometry to ascertain if a hybrid has been accomplished, but many small-scale breeders do not have access to such expensive and technical hardware. Can a specific hybrid be determined with less complex technologies? In general, the answer is yes, although with the caveat that such methods are not truly definitive but do point to a particular outcome. Bear in mind that a negative result tells nothing, but a positive result indicates something did occur.

One of the first indicators that a successful cross has been made is that resultant fruit or seed capsule is misshapen, or irregular compared to the norm for the mother plant. Along these lines is the degree of fill of seeds in a given fruit, if there are gaps and chambers are sparse or not filled the likely hood is that some form of cross pollination did take place. It should be remembered that the mother plant exercises a great many controls on pollination and seed development and some seed will be absent due to maternal influence. For instance, in a wide cross of *Hibiscus palustris* 772 with *H. coccinea* 45, the fruit is smaller, and the seed count is greatly reduced, 105 for the norm verses 43 for the cross. This is almost definitive that a successful pollination did take place.

Figure 3. Partially filled *Hibiscus* fruit.

Figure 4. Variable fruit size in normal and hybrid *Hibiscus* crosses.

Another indicator of successful hybridization is relative seed size. In the *Hibiscus* hybrid seed is often smaller and, in some cases, misshapen. The exact cause of this is not known but a good assumption is that the hybrid seed is under stress by the mother plant and development is slower than that of seed which is more normal. Fig. 8 shows the disparity with hybrids of *Hibiscus aculentus* with a wide cross of another *Hibiscus* species.

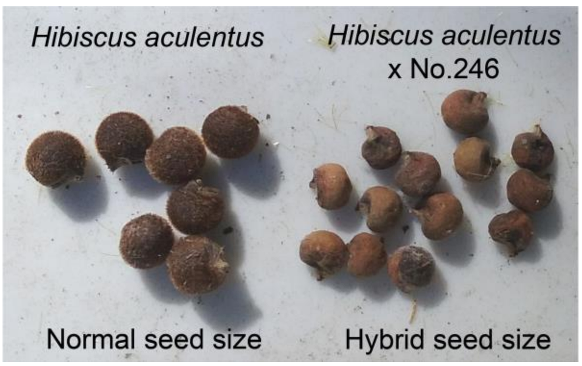

Figure 5. Seed size as a clue indicating a successful hybrid pollination in *Hibiscus*.

Other morphological and physiological changes to detect hybrids

- 1. Seed is good but refuses to germinate
- 2. Seed is good but germination stratification and time is altered
- 3. very low seed germination but a clear sign of a hybrid
- 4. seedlings appear to be weak or deformed and slow to begin growing well

Figure 6. Morphological and physiological indicators of successful hybridization.

There are other indicators of a successful hybrid being formed. Sometimes juvenile leaf morphology is changed from the norm but might revert upon more mature leaves forming. In some instances, the seedlings will not grow straight away compared to those of a normal pollination. Cold hardiness in the case of cold tolerant plants could well be affected and those with a dramatic new genetic constitution might not be as hardy as before. It is reasonable to assume the cross of a non-hardy plant with a hardy plant will result in less hardiness. This is not necessarily the case and often depends which was the mother plant. In many cases the mother plants genes have a dominant effect on the seedlings and seedlings will commonly behave like the mother plant.

Conclusion

The variations that are present in hybrid seedlings can be extensive and not always easily seen. Part of the process of selection and testing is to uncover the hidden traits. Sometimes this is a rather quick process, leaf color or morphology can quickly tell which is which. For instance, *Cercis canadensis alba* seedlings can be quickly discerned from those of the pink forms by a difference in the colors of the leaf petioles. Telling one pink from another is not such an easy task. Leaf morphology and seed germination times also might indicate fundamental differences. Cold, heat or salt tolerance can be tested reasonably easily, and the results can be dramatic, a large block of seedlings can quickly be reduced to a mere few. With the advent of new and much superior genetic testing a high value plant might be easily and quickly selected over those of less stature.

There is hope for the small breeder regardless. Small breeders can concentrate on specific plants not in the main stream. Small breeders can also look for traits that might be lost in the fog for a large breeder. Small breeders might not have the resources to go after genetically modified plants that seem to incur a great deal of ire from certain segments of the buying public. Finally, small breeders might be more adept at producing specific clones and new hybrids that are regionally adapted. At present there is room for all with enough initiative to tackle new projects and species not found in the main stream.

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