The Basics of Plant Hybridization and Improvement©

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INTRODUCTION

New ornamental plant cultivars can arise in any number of ways, including discovery and domestication of desirable plants found in the wild, identification and commercialization of superior seedlings obtained from seed collected off of garden-worthy plants, and rare shoot or bud sports arising on pre-existing cultivars. Additionally, many new cultivars of plants have arisen as a consequence of controlled hybridization between selected parents. Although successful development of new, improved plant cultivars by controlled hybridization is maximized if the hybridizer possesses a solid foundation in plant genetics, amateur plant breeders can and have had significant success in the development of new ornamental cultivars. In this presentation, some of the basic principles and techniques involved in plant hybridization and improvement will be discussed. This background will equip the amateur with the tools to initiate a simple plant breeding effort. The presentation will focus on the following steps in the hybridization program.

HYBRIDIZATION PROGRAM

Selection of the Appropriate Parents. Parents used in hybridization are typically chosen based on combining different desirable ornamental traits found in each parent into an individual plant resulting from hybridization.

Synchronizing Chosen Parents to Flower Simultaneously. It is not uncommon in hybridization efforts for the breeder to be faced with the problem of asynchrony of flowering between the two chosen parents. If the parents being hybridized are maintained in pots, it is possible to attempt to manipulate temperature or photoperiod (long-day, short-day conditions) to synchronize flowering between the parents.

Pollen Collection. If routine cultural manipulation, as described above, is unsuccessful in synchronizing flowering between the two parents, one can resort to pollen collection and subsequent short- or long-term storage of pollen from the male parent. This is commonly done with many species to facilitate hybridization between two parents with asynchronous flowering.Even in cases where the chosen parents can be flowered at the same time in the greenhouse, or in cases where hybridization is conducted outside, prior collection and storage of pollen from the proposed male parent, rather than having to collect pollen at the time of actual crossing — often can increase hybridization speed and efficiency. Pollen can be acquired by collecting flower buds from field plants, about 1 or 2 days prior to anticipated flower anthesis. Alternatively, if one is working with spring-flowering woody shrubs or trees, one can collect shoots from field plants 1 to 2 weeks prior to anticipated time of flowering in the field. Shoots can be forced indoors in a florist's preservative solution until flower buds reach the appropriate stage of development. Anthers, the pollen bearing organ, can then be removed from the flower buds by hand, by excising with a scissors, or by rubbing the tip of the flower buds gently over the surface of common window screen mesh. Anthers

should be placed on a piece of absorbent paper and allowed to dry at room temperature for 24 to 36 h, preferably at low relative humidity, during which time the anthers will dehisce and release the pollen. The dried anthers and pollen can then be transferred to a tightly closed glass or plastic vial. The vial of pollen then can be stored in an ordinary household freezer for a prescribed period of time, depending on the species and the storage conditions. When the later flowering female parent comes into flower, the pollen can be transported to the field or greenhouse in a ice chest to keep it cold, and used in the hybridization process. Stored pollen removed from the freezer should be returned as quickly as possible after use to the freezer to maximize its longevity and future use.

Short- and Long-Term Storage of Pollen, and Prediction of Storage Longevity. Pollen storage is not possible with all plant species. In many species, such as those in the genus *Prunus*, pollen can be stored for 1 to 2 years with little loss of viability. In other species, such as those in the family Compositae (Asteraceae), and in the grasses, pollen longevity is very short, often measured in days rather than years, and storage is generally impractical. How can one predict longevity? Plant species are classified as possessing either binucleate or trinucleate pollen, referring to the number of nuclei the pollen grain possesses when it is shed from the anther. In general, those species possessing binucleate pollen can be successfully stored for long time periods, while those possessing trinucleate pollen demonstrate poor storage longevity. Approximately 75% and 25% of plant species possess binucleate and trinucleate pollen, respectively.

Importance of the Direction of the Cross. The direction of the cross refers to the choice of which plant is chosen as the male or female parent. Generally, direction of cross makes little difference as regards the genetic consequences of the hybridization. Certainly, one should choose the more fruitful plant as the female parent if known differences in seed set potential between the parents are known. In some cases when a cross is made with a variegated parent, the variegated plant should be used as the female parent to increase the likelihood of transmission of the variegated trait. However, variegation is a complex trait, and often does not exhibit genetic transmission from parent to offspring, regardless of cross direction. In cases where a hybridization is made between a white-flowered and a pigmented-flowered parent, it is advisable to use the white-flowered plant as the female parent. Because pigmentation in flowers is usually genetically dominant to nonpigmentation, the true hybrids from such a cross can be identified easily by observing flower color; white-flowered offspring will have resulted from self pollination, and should be discarded, while pigmented offspring are likely true hybrid progeny, assuming the pigmented parent is genetically homozygous (true breeding) for pigmentation. Breeders refer to these dominant traits that are useful in confirming hybridity of progeny as genetic markers. Other known dominant traits can also be used as genetic markers in crosses.

Emasculation (Removal of Male Flower Parts from Female Parent) Procedures. In self-pollinating species, removal of the anthers, the pollen bearing organs, prior to flowering, is necessary on the female parent to prevent the risk of self pollination and contamination of the hybridization. Anthers can be conveniently removed using fine forceps. On species with small flowers, magnifying lenses are often helpful. Anthers should be removed a day or two prior to anticipated opening of the

flower. Good practice dictates that minimal injury be imposed on the remaining flower parts during emasculation. Such injury often negatively impacts hybridization success. With cross-pollinating species, emasculation often is not required. Many cross-pollinating species demonstrate a reproductive characteristic called self-incompatibility. Plants of self incompatible species make functional pollen and egg cells, but they are incapable of setting seed from self pollination, or from pollen transfer between different plants of the same cultivar. If one is breeding a self-incompatible plant, emasculation is not necessary. In this case, one can simply obtain pollen from the male parent using a camel's hair brush and transfer it to the flower of the female parent. Self-incompatibility will dictate that any seeds produced primarily will be from cross pollination. In some self-incompatible species, minimal amounts of selfing will occur. However, because self-incompatible species often demonstrate inbreeding depression, plants arising from selfing often are weaker and inferior to those plants derived from outcrossing, and can be recognized and discarded.

Pollination of the Female Parent and Protection of Pollinated Flowers. At the time the emasculated flower on the female parent is receptive, pollen can be deposited on the stigmatic surface. The "window of receptivity" for a flower varies depending on species, and is best determined through trial and experience. Pollen can be effectively transferred from the male parent flower or from previously collected pollen stored in a vial to a flower on the female parent using a camel's hair brush. Generally a #2 brush works well for many species. Dip the brush in a solution of 70% ethyl or isopropyl alcohol between different hybridizations to kill pollen and prevent contamination between different crosses. Each flower should be tagged and labeled with the specific cross and the date of hybridization. Use a pencil or water-fast pen to ensure permanence of the record. If the female parent is grown in a location where subsequent insect visitation may contaminate the flower with foreign pollen, it is advisable to cover the flower to prevent insect visitation. Any woven materials, such as cheesecloth or panty-hose, that allows air exchange but excludes pollinators are appropriate.

Observation of Fruit Set and Development. After hybridization, success of the cross can often be judged by the visible swelling of the fruit. Fruit should be allowed to develop until full maturity. Fruit should be allowed to dry on the mother plant as much as possible, but examine plants regularly to ensure that seeds are not expelled from the fruit prior to collection.

Collection of Fruit and Extraction of Seeds. Collect fruit from the female parent at maturity, extract the seeds, and dry the seeds if necessary. Drying would not be recommended on those species where desiccation reduces seed viability. Seed should be stored in conditions appropriate for the particular species.

Seed Germination. Seed should be germinated using procedures appropriate for the species.

Seedling Culture and Transplanting to Field or Garden Site. Hybrid progeny should be transplanted to the field or greenhouse site and grown using standard cultural procedures.

Field Selection among Hybrids. Selections should be made based on the traits of interest for the particular cross. Selected plants should be flagged or staked.

Plants not deemed useful should bediscarded. In later stages of testing, inclusion of named cultivars should be included as standards for comparison.

Remember Mendel! Many amateur hybridizers err in forgetting the laws of the father of genetics — Gregor Mendel. They fail to generate the second generation of progeny from a cross. Hence, the hybridizer doesn't take advantage of the fact that recessive traits are expressed, and most traits segregate and recombine independently, in the second generation. The practical implication of this is that the hybridizer may not recover the desired offspring in the first (F_1) generation derived from the cross, but such offspring may be obtained in the second (F_2) generation. Thus, one is required to select the better progeny in the F_1 generation, isolate them from foreign pollen of plants that are sexually compatible, and allow for the production of F_2 seed. With self-fertile species, flowers on an individual F_1 plant or multiple plants can simply be covered to exclude insect visitation and contamination. Cross pollination species are incapable of setting self seed, hence a population (10 to 15 plants minimum) of F_1 progeny derived from the same hybridization must be intermated by hand pollination, taking measures to exclude pollen contamination by insects. Alternatively, the F_1 individuals to be intermated can be grown in an isolated location at least 200 yards from any other plants that are sexually compatible, allowing insects to cross pollinate the plants. If isolation in the field is impossible, another choice is to grow the F_1 progeny in pots, place them in a screened enclosure, and introduce a hive of honeybees or a nest of bumblebees into the cage during flowering.

Propagation of Selected Seedlings. Final selections should be propagated using the appropriate procedure, and tested over 2 to 4 years, preferably at more than one location, to verify garden performance and superiority.