Polyploidy: From Evolution to New Plant Development©

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INTRODUCTION
Polyploidy is an intriguing phenomenon in plants that has provided an important pathway for evolution and speciation. Although the first polyploid was discovered over a century ago, the genetic and evolutionary implications of polyploidy are still being elucidated (Bennett, 2004; Soltis et al., 2003). On a more practical level, there are many opportunities for utilizing polyploidy as a valuable tool in traditional plant breeding programs.

WHAT IS POLYPLOIDY AND HOW DOES IT ARISE?
A polyploid is simply an organism that contains more than two complete sets of chromosomes. For animals, this is a fairly rare occurrence (though a polyploid rat, the first polyploid mammal ever identified, was recently discovered in Argentina). In plants, however, polyploidy occurs naturally and is very common.

The term "ploidy" or "ploidy level" refers to the number of complete sets of chromosomes and is notated by an "x." An individual with two sets of chromosomes is referred to as a diploid (2x), three sets would be a triploid (3x), and so on with tetraploid (4x), pentaploid (5x), hexaploid (6x), etc. It is sometimes also important to identify if one is referring to the reduced (gametophytic) chromosome number following meiosis as would be found in egg and sperm (denoted as "n") or in nonreduced (sporophytic) tissue (denoted as "2n"). Thus, for example, a tetraploid birch tree would be presented as 2n = 4x = 56.

Polyploidy can naturally arise in a number of different ways. In some cases a somatic (nonreproductive) mutation can occur, due to a disruption in mitosis, resulting in chromosome doubling in a meristematic cell(s) that will give rise to a polyploid shoot. These sports are sometimes evident on a plant by their enlarged "gigas" condition. Polyploids can also result from the union of unreduced gametes — eggs and sperm that have not undergone normal meiosis and still have a 2n constitution.

The origin of a polyploid can often determine if it will be fertile and may further indicate how it can best be used in a plant improvement program. If a tetraploid arises from spontaneous doubling in a shoot or from the union of unreduced gametes from two closely related (e.g., same species) diploid individuals, it will have four similar (homologous) versions of each chromosome. Despite different origins, both of these polyploids behave similarly reproductively and are often referred to as autotetraploids (or polysomic tetraploids). Autopolyploids may or may not be fertile. In diploids, meiosis involves the pairing of homologous chromosomes, which eventually segregate to form two separate gametes, each with one set of chromosomes. Infertility can arise in autopolyploids due to the fact that there are more than two homologous chromosomes. The presence of multiple homologous chromosomes often results in spurious pairing between multiple chromosomes, unpaired chromosomes, and gametes with unbalanced chromosome numbers (anueploids).

Offspring that result from sexual reproduction from unreduced gametes or somatic doubling in a hybrid of different species are referred to as allopolyploids (or sometimes amphidiploids or disomic polyploids). These plants also have four versions of each chromosome, but the two from one parent are sufficiently different from the two from the other parent, that they generally don't pair during meiosis. Due to this composition, allopolyploids are typically fertile. During meiosis each chromosome can pair with its homologous partner, meiosis continues, resulting in fertile germ cells.

In many cases polyploids fall somewhere in between an autopolyploid and allopolyploid whereby there is partial chromosome homology resulting in a combination of disomic and polysomic pairing and are referred to as segmental allopolyploids.

ROLE OF POLYPLOIDS IN PLANT EVOLUTION

In contrast to the gradual evolutionary process whereby new species evolve from isolated populations, new species of plants can also arise abruptly. The most common mechanism for abrupt speciation is through the formation of natural polyploids. Once a tetraploid arises in a population, it can generally hybridize with other tetraploids. However, these tetraploids are reproductively isolated from their parental species. Tetraploids that cross with diploids of the parental species will result in triploids that are typically sterile. This phenomenon provides a "reproductive barrier" between the polyploids and the parental species — a driving force for speciation.

Various estimates suggest that as many as 47%–70% of flowering plants are of polyploid origin (Grant, 1971; Goldblatt, 1980; Ramsey and Schemske, 1998). For example, the plants in the rosaceous subfamily maloideae (*Malus, Pyrus, Photinia, Chaenomeles*, etc.) are believed to have originated from ancient allopolyploids since they have $n = 17$ base chromosomes whereas plants in other rosaceous subfamilies have $n = 8$ or 9 (Rowley, 1993). In many genera, different species will have different ploidy levels (multiples of a base number) representing a series of polyploids. In the genus *Chrysanthemum* (syn. *Dendranthema*), different species have chromosome numbers of 2n = 18, 36, 54, 72, 90, and 198 — all multiples of a base chromosome number of 9.

There are a number of factors that may provide polyploids with adaptive and evolutionary advantages. Perhaps most importantly, polyploids can be significantly more heterozygous than their diploid counterparts. Polyploids can have 4 different genes (alleles) present at any given locus (location on a chromosome). The degree of heterozygosity may be a key factor in the growth, performance, and adaptability of a polyploid. Allopolyploids can have a much greater degree of heterozygosity (dissimilar genes) which can contribute to heterosis or hybrid vigor. Furthermore, this heterozygosity is somewhat fixed (chromosomes that originated from a given species preferentially pair with similar homologous chromosomes during meiosis, ensuring that the genomes of both parental species will continue to be present). On the other hand, the addition of multiple copies of homozygous chromosomes (as would be the case with autopolyploids), does little to enhance genetic superiority and can actually reduce vigor and fertility by creating a more "inbred" situation.

Since all polyploids have a certain amount of genetic redundancy, extra copies of genes can mutate and diverge resulting in new traits without compromising essential functions. Polyploid populations often demonstrate extensive genomic rearrangement including the origin of novel regions of DNA (Arnold, 1997; Song

et al., 1995; Wendel, 2000). Ancient polyploids can eventually undergo such changes to the extent that they effectively become "diploidized" where diploid gene ratios are restored.

Polyploids also tend to be more self-fertile (not requiring cross pollination) and apomictic (producing seeds with embryos derived directly from maternal tissue, not sexual fertilization). Since polyploids generally arise at a low frequency, greater self fertility and apomixis would help to compensate for their minority-disadvantage (Briggs and Walters, 1977) and would provide further benefits in areas where breeding systems are compromised in stressful environments. Furthermore, inbreeding is less deleterious for allopolyploids due to their greater heterozygous nature.

One question that frequently arises is whether or not polyploids inherently have greater stress tolerance. For example, it has often been observed that disproportionate number of polyploids are found in cold, dry regions. Some argue that this is a spurious correlation (Sanford, 1983) or possibly the result of intermixing of species and formation of allopolyploids during glacial periods (Stebbins, 1984). However, polyploids may also have certain characteristics that do provide some adaptive benefits. Molecular studies have demonstrated that allopolyploids exhibit "enzyme multiplicity" (Soltis and Soltis, 1993). Since allopolyploids represent a fusion of two distinctly different genomes, these polyploids can potentially produce all of the enzymes produced by each parent as well as new hybrid enzymes. This enzyme multiplicity may provide polyploids with greater biochemical flexibility; possibly extending the range of environments in which the plant can grow (Roose and Gottlieb, 1976). Other changes in gene expression, altered regulatory interactions, and rapid genetic and epigenetic changes could further contribute to increased variation and new phenotypes (Osborn, et al., 2003).

POLYPLOIDY AND PLANT IMPROVEMENT

Considering the profound importance of polyploidy in plant evolution, it is understandable that there was considerable interest in developing induced polyploids when mitotic inhibitors were first discovered in the 1930s. However, despite the fact that polyploids have been developed for many major crops, these plants are almost always found to be inferior to their diploid progenitors. Somatic doubling does not introduce any new genetic material, but rather produces additional copies of existing chromosomes. This extra DNA must be replicated with each cell division. Enlarged cell size is often associated with polyploids, which can result in anatomical imbalances. Other deleterious effects can include erratic bearing, brittle wood, and watery fruit (Sanford, 1983). High-level polyploids (e.g., octaploids) can be stunted and malformed, possibly resulting from the extreme genetic redundancy and somatic instability that leads to chimeral tissue.

Despite the drawbacks of induced autopolyploids, these plants may be valuable if they are in turn used in a breeding program to enhance the degree of heterozygosity and and further selected for desirable traits. In most cases it appears that inducing autopolyploids will do little for plant improvement unless substantial heterozygosity can be incorporated (Sanford, 1983). Historically, work with polyploids has not progressed much beyond somatic doubling — resulting in considerable genetic redundancy. Based on our knowledge of natural systems and evolution, it appears that much greater advances can be made by working towards enhanced heterozygosity, including the development of allopolyploids.

Polyploidy can result in a wide range of effects on plants, but the specific effects will vary dramatically based on the species in question, the degree of heterozygosity, the ploidy level, and the mechanisms that relate to gene silencing, gene interactions, gene dose effects, and regulation of specific traits and processes.

OPPORTUNITIES

Overcoming Barriers to Hybridization. In some cases, desirable crosses are difficult to obtain due to differences in ploidy levels between prospective parents. Such interploid barriers appear to arise from abnormal endosperm formation. In species where there is an interploid block, seeds will often only develop normally if there is a 2 maternal : 1 paternal ratio in the genomic makeup of the endosperm, which would be the normal case for two diploid parents (Ramsey and Schemske, 1998). Seeds that don't meet this criterion are often underdeveloped or abort. In some cases this ratio is not exact, but the greater the disparity, the lower the viability of the seeds (Sanford, 1983). In cases where interploid blocks exist, barriers to hybridization may be overcome by manipulating the ploidy levels to match prior to hybridization.

Developing Sterile Cultivars. The introduction and movement of invasive species can be a significant threat to certain ecosystems. Development of sterile forms of important nursery crops is an ideal approach for addressing this problem. In doing so, plants can be grown and used for landscaping while minimizing the possibility that these plants could sexually reproduce and become invasive. There are a number of methods available for developing sterile plants. However, one of the most rapid and cost-effective approaches for inducing sterility in a plant is by creating polyploids. In most cases these plants function normally with the exception of reproduction, specifically meiosis. In some cases doubling the chromosomes of an individual plant (autotetrapoid) will result in sterility due to multiple homologous chromosomes and complications during meiosis (as discussed previously). Despite these complications, autotetraploids of some species can produce fertile seeds. In this case, tetraploids can then be hybridized with diploids to create sterile triploids. Triploids have an additional reproductive barrier in that the 3 sets of chromosomes cannot be divided evenly during meiosis yielding unequal segregation of the chromosomes (aneuploids). Even in the unusual case when a triploid plant can produce a seed (apples are an example), it happens infrequently and seedlings are usually abnormal.

Development of triploids of some species can be complicated due to the presence of an interploid block that prevents the normal development of triploid embryos. However, embryo culture is an additional technique that can be employed to overcome this problem and produce sterile triploid plants.

An alternative approach for creating triploid plants is regeneration of plants from endosperm found in seeds. Although the embryo in most angiosperm seeds is diploid, the adjoining endosperm (nutritive tissue) originates from the fusion of three haploid nuclei (one from the male gametophyte and two from the female) resulting in triploid tissue. This tissue can be excised from developing seeds and cultured in vitro (tissue culture) to eventually give rise to regenerated embryos and plantlets. This approach has been successful for a range of plants including citrus, kiwifruit, loquat, passionflower, acacia, rice, and pawpaw.

Restoring Fertility in Wide Hybrids. It is not unusual for hybrids between distant taxa (different species or genera) to be sterile. This often occurs due to failure of the chromosomes to pair correctly during meiosis — referred to as chromosomal sterility. By doubling the chromosomes of a wide hybrid, each chromosome has an exact duplicate and chromosomal homology and fertility can be restored. This technique has been used successfully to restore fertility in *Rhododendron* 'Fragrans Affinity' and ×*Chitalpa tashkentensis* (Contreras, 2006; Olsen, 2006). However, in some cases this approach has been unsuccessful in restoring fertility, as was the case with tetraploid hybrids of *Alstroemeria aurea* × *A. caryophyllaea* (Lu and Bridgen, 1997).

Enhancing Pest Resistance and Stress Tolerance. The influence of polyploidy on adaptability and resistance to biotic and abiotic stresses has been widely studied in crop plants (Levin, 1983). In some cases polyploids have demonstrated greater resistance to pests and pathogens, greater nutrient uptake efficiency, better drought resistance, and superior cold tolerance. However, polyploidy often results in reduced resistance to these same stresses as well. It should not be assumed that polyploids are necessarily more stress tolerant.

   There are a number of strategies for inducing polyploids as a means of enhancing adaptability. Increasing the chromosome number and related gene dose can sometimes enhance the expression and concentration of certain secondary metabolites and defense chemicals. However, this is not always the case and little is generally known about the relationship between gene dose, gene silencing, and expression of secondary metabolites. A more promising approach would be to create allopolyploids between plants with diverse endogenous secondary metabolites. A unique and valuable characteristic of allopolyploids is that the secondary metabolites from the parental species are typically additive. That is to say that allopolyploids often produce all the enzymes and metabolites (including defense chemicals) of both parents. This could be particularly effectively for combining the pest resistant characteristics of two species, and potentially contributing to a much broader, more horizontal form of pest resistance. A similar approach may have utility for enhancing tolerance to certain environmental stresses.

Enlargement and Enhanced Vigor. Although enlarged cell size found in some polyploids can have undesirable effects, it can sometimes also be beneficial. In some plants, polyploidy results in significant enlargement. Fruit from tetraploid apples can be twice as large as diploid fruit, though they tend to be watery and misshapen. For apples, triploids have proven to be a happy medium that combines larger fruit while retaining good quality and are often grown for commercial production. This type of enlargement can be particularly desirable for ornamental flowers. Flower petals can also be thicker and flowers can be longer lasting in polyploid plants (Kehr, 1996).

METHODS FOR INDUCING POLYPLOIDY

In the late 1930s it was discovered by that colchicine inhibits the formation of spindle fibers and temporarily arrests mitosis at the anaphase stage (Blakeslee, 1937). At this point, the chromosomes have replicated, but cell division has not yet

taken place resulting in polyploid cells. Other mitotic inhibitors, including oryzalin, trifluralin, amiprophos-methyl, and N_2O gas have also been identified and used as doubling agents (Bouvier et al., 1994; van Tuyl et al., 1992; Taylor et al., 1976).

Methods for applying these agents vary. One of the easiest and most effective methods is to work with a large number of seedlings with small, actively growing meristems. Seedlings can be soaked or the apical meristems can be submerged with different concentrations, durations, or frequencies of a given doubling agent. Shoots on older plants can be treated, but it is often less successful and results in a greater percentage of cytochimeras. Treatment of smaller axillary or sub-axillary meristems is sometimes more effective. Chemical solutions can be applied to buds using cotton, agar, or lanolin or by dipping branch tips into a solution for a few hours or days. Surfactants, wetting agents, and other carriers (dimethyl sulfoxide) are sometimes used to enhance efficacy.

VERIFYING POLYPLOIDY LEVELS

Plants with increased ploidy levels are sometimes apparent by their distinct morphology. Increasing ploidy often results in increased cell size that in turn results in thicker, broader leaves and larger flowers and fruit. Shoots are often thicker and can have shortened internodes and wider crotch angles. Plants with high ploidy levels (e.g., octaploids) can have distorted growth and reduced growth rates. When screening large numbers of plants, these visual characteristics are sometimes helpful for identifying putative polyploids. Other effective, but more time-consuming, measures that indicate polyploidy include larger pollen size, greater number of chloroplasts per guard cell (Solov'eva, 1990), and larger guard cells and stomates. Flow cytometry is a very useful tool for measuring DNA content which can be correlated with ploidy level for a given crop (Sharma and Sharma, 1999). Traditional cytology is often necessary to determine chromosomes number and ploidy level. Techniques include measurements on young leaves, root tips, and anthers (Ruzin, 1999).

When testing and breeding polyploids, it is important to recognize that induced polyploids can sometimes be cytochimeras where the ploidy level varies in different types of tissue. Meristems are typically divided into three histogenic layers L-1, L-2, and L-3. Mutations and doubling agents may result in increased ploidy levels in one, two, or all three layers. For information on reproductive behavior, it is important to measure the ploidy level of L-2, or cortical layer, which is reflected in pollen size and chromosome counts from reproductive tissue (e.g., anthers). Root tips would reflect the L-3 layer while the guard cells would reflect the L-1 layer.

SUMMARY

In the vast majority of cases, induction of autopolyploids will not, in of itself, result in substantially improved landscape plants. However, with knowledge of the origins of, variations in, and characteristics of different types of polyploids, there are many opportunities for developing and utilizing polyploids in plant improvement programs. Significant opportunities include developing sterile cultivars, overcoming barriers to hybridization, restoring fertility in wide hybrids, enhancing flower size, increasing heterosis and vigor, and improving pest resistance and tolerance to environmental stresses.

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