Wide Crosses In Plants Where Results Are Similar To *Medicago sativa* X *M. arborea* Crosses

Edwin Bingham, email: ebingham@wisc.edu

Interspecific and intergeneric hybridization does not always result in balanced hybrids with the chromosomes of both parents. Often there are changes such as chromosome elimination, chromosome duplication, chromosome fragment loss or gain, gene inactivation, gene activation, and activation of transposable elements (see literature reviews in the following references). There seems to be precedence in the literature for every possible outcome. Herein, we cite examples of DNA introgression and trait transfer in wide crosses without balanced hybridization. This appears to be the case in the sexual hybrids of *Medicago sativa* X *M. arborea* produced in Wisconsin, and in Australia. We have known for five years that *M. arborea* traits have been transferred. More recently, it has been shown that hybrids contain *M. arborea*-specific DNA . No two progeny are alike in fertility or morphology, and most contain different *M. arborea*-specific DNA bands. Thus, individual hybrids have pieces of the *M. arborea* genome, and the complete genome could be spread over the ca 20 hybrids on hand. This appears to be an advantage in backcrossing desired traits, because linkage drag is minimized.

A study of intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus* by Cheng et al. 2006 (Genome 45:110-115), reports unbalanced hybridization similar to that in our *Medicago* hybrids, and reviews pertinent literature in *Crepis, Hordeum, Helianathus*, and *Brassica*. The *Brassica* X *Orychophragmus* hybrids varied widely and were studied in three groups: one group with less than the expected hybrid chromosome number, and two groups with more than the expected number. Results were explained by chromosome doubling followed by chromosome elimination during seed development.

The following paper is a rich source of references, and can be found on the web by searching for the title: Alien DNA Introgression and Wheat DNA Rearrangements in a Stable Wheat Line Derived from the Early Generation of Distant Hybridization, by Zhang et al. 2005, Science in China, vol. 48. The title essentially summarizes the paper. In a study by Zhou et al. 1979 (Acta Genet. Sinica 6:405-413), the architecture of rice was changed by crossing it with sorghum. They used large amounts of sorghum pollen mixed with a small amount of rice pollen. They argue that the preponderance of sorghum pollen tubes and sperm nuclei in intimate contact with the fertilization process somehow transferred DNA.

Intergeneric hybrids of *Tripsacum* species (2n=36) crossed with *Zea mays* (2n=20, are difficult to produce, de Wet et al. 1984 (Amer. J. Bot. 71:245-251). Nonetheless, several hybrids were obtained in studies over several years. Most hybrids involved gametes from both parents, although some gametes were non-reduced, and resulting hybrids had various chromosome combinations from both parents. However, there was a class of hybrids morphologically distinct from the *Tripascum* parent, but lacking *Zea* chromosomes. These hybrids had traits of both parents, but possesed only the chromosomes of the *Tripsacum* seed parent. Interestingly, *Zea* genetic material appeared introgressed into *Tripsacum* chromosomes, with no effect on the karyotype. This paper cites other unusual wide hybrids in *Gossypium, Hordeum*, and *Petunia,* and discusses heterofertilization in maize. George Sprague discovered heterofertilization in maize in 1929, and it has been researched extensively since that time, as evidenced by dozens of web sites. Heterofertilization is proof that unusual things can happen in the process of sexual reproduction.

In *Medicago*, protoplasts of *M. rugosa* and *M. scutallata*, both annuals with 2n=30, were electrofused with protoplasts of M. sativa 2n=32, and plants regenerated from both combinations by Mizukami et al. 2006 (Plant Cell Tissue Organ Cult. 84:80-89). Hybrids were confirmed by morphology, parental specific bands, and cytology. Coexpression of yellow and purple parental flower colors was vivid evidence of hybridity. The expected somatic chromosome number was 60, but original somatic hybrids were aneuploids with 2n=31-59. During plant growth and development, however, their chromosome number was reduced to 32, the normal number of chromosomes for alfalfa.   The hybrids resembled alfalfa, and were perennials, however, in situ hybridization showed chromosome rearrangements and introgression of DNA from both annual *Medicago* species. This paper reviews other interspecific hybrids in *Medicago* made sexually and by somatic fusion, including electrofusion somatic hybrids of *M. sativa* and *M*. *arborea* produced by Nenz et al. 2006 (Theor. Appl. Genet. 93:183-189).

In the case of *M. sativa* X *M. arborea* sexual hybrids, reported on this web site in volumes 5,7and 9, we have observed that individual hybrids express one to several *M. arborea*-specific traits involving flower color, size of leaves, stems and seed, but no individual hybrid appears to be half *M. arborea*. The ratio of DNA bands specific to each parent shows this also. The most complete knowledge about sexually produced hybrids of *M. sativa* X *M. arborea* is from Armour et al. 2008 (Theor. Appl. Genet. 117:149-156).   These Australian researchers produced five hybrids, and showed that anthracnose resistance and unusual pod coiling had been transferred.   In the most studied hybrid, M. sativa DNA bands were most prevalent, 4 percent of the bands were unique to *M. arborea*, and there were bands not present in either parent, suggestive of chromosome rearrangements. Their hybrids were at or near 2n=32 chromosomes, and they suggested hybrids may be the result of fertilization of non-reduced eggs of the M. sativa parent by normal gametes of *M. arborea*, followed by elimination of most of the *M. arborea* chromosomes, except for introgression of pieces into the *M. sativa* chromosomes.

The literature search is ongoing, but at this point, it appears that in wide crosses, unbalanced hybrids are the rule rather than the exception. In our case, we are continuing to study how hybrids of *M. sativa* X *M. arborea* are produced. However, as mentioned in an earlier report, we can exploit materials in plant breeding without knowing the basic mechanisms. Fortunately, we started the breeding process about five years ago, and several hybrid selections are ready for testing in crosses with alfalfa.