Research on the Mechanism of Genetic Transfer from Medicago arborea to Alfalfa

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The first sexual hybrids of alfalfa X M. arborea were reported on this web site in 2005, and since that time, we have been trying to understand how hybrids are produced. Eventually, we focused on the male sterile clone M8 that is the most efficient hybridizer. Clone M8 is from a three-way X three-way cross involving M. sativa subspecies sativa, coerulea, and falcata (see first report in vol. 9). All of the subspecies parents had normal fertility; hence the male sterility of M8 is due to hybrid breakdown. Pollen does not appear to be shed when tripping M8 flowers, but if hundreds of flowers are tripped onto a pollen applicator, two or three self seeds can be produced per hundred flowers. By 2009 we had concluded that M8 self pollen was somehow involved. What role does the trace amount of self pollen play? Does it mentor M. arborea pollen, or is it something more complicated?

Then, in early 2010, we discovered that M8 was capable of haploid apomixis; three haploids were confirmed among 40 self progeny of M8. Details of this will be reported later, but the discovery of apomixis indicated that the female gametophyte is abnormal. An abnormal female gametophyte could weaken the barrier to wide crosses. Once it was clear that the female gametophyte was abnormal, it was equally clear that the male gametophyte was also abnormal. Nearly all male gametophytes of M8 fail to develop. Are abnormal gametophytes enabling the wide cross? If so, is it the female, the male, or both? The challenge is to design crossing experiments to answer these questions.

The "Mixomedic" Project and the Origin of Clone M8

Introduction. In 1995 a number of diverse Medicago sativa subspecies materials were crossed to use as resources in our genetic toolbox. The most diverse and unrelated materials on hand were diallel crossed, and then diallel crosses were used in three-way crosses. This was labeled the Mixomedic Project. At the time, we had no idea that the subspecies were diverse enough to cause hybrid breakdown in the advanced generations, nor did we plan to use the material in wide crosses.

The last cross was made in the greenhouse on a cold January morning in 1996, when three-way hybrid plants were growing next to each other in the greenhouse, and a three-way X three-way cross was made, "just because it was there". The least self fertile plant was used as the seed parent, and one raceme was pollinated with pollen from the other plant. The seed went into storage in 1996, where it stayed until we found the time to examine the cross in 1999. The 33 seeds from the cross were planted in the greenhouse, and 28 surviving plants were transplanted to a field nursery. Information

from our field book is reported below. Plant 5 was cloned and saved because it produced n, 2n, and 4n pollen (see report in vol. 5 of Medicago Genetic Reports), and plant 8, now M8, was cloned and saved because it was male sterile (see vol. 9). Plant M8 also had cream colored flowers that now figure prominently into our research, but at the time we were only curious about the male sterility, because all of the parents had normal fertility.

Materials. Six parents; all tetraploids; all normal in male and female fertility; all trifoliolate; all typical of their respective subspecies.

1. M. falcata-a, selected from WISFAL, dark yellow/orange flower color, tall and robust, fall dormant.
2. M. sativa-Non Dormant (ND), selected from Wadi-Qurat, purple flower color, pubescent, winter active.
3. M. falcata-c, similar to number 1 above, except a tree-like, strong main stem.
4. M. coerulea, tetraploid via colchicines doubling, obtained from R. P. Murphy, Cornell Univ., low growing, purple flower color.
5. M. falcata-c, similar to number 1 above, except larger seed.
6. M. sativa-Dormant (D), selected from cultivar 'Columbia 2000', purple flower color, self fertile, fall dormant.

Structure of the 3-way X 3-way cross giving rise to clone M8.

[(M. falcata-a X M. sativa-ND) X M. falcata-b] **X** [(M. coerulea X M. falcata-c) X M. sativa-D]

All parents had normal fertility, i.e. normal pollen and normal seed set of 4-6 seeds per pod. All parents had normal leaves, i.e. normal trifoliolate leaves; no multifoliolate leaves were noted.

Field observations of 28 subspecies hybrid plants from the 3-way X 3-way cross.

Flower color: Expect: 2-3 cream/light yellow (ca 10%). Observed: 9 cream/light yellow (ca 30%). Hence, there was segregation distortion at the P locus.

Three aneuploids were confirmed (two 2n=30; one 2n=31), and there could have been more. Hence, 10% aneuploids. Expected background aneuploid frequency in alfalfa is 3%.

Abnormal pollen; not one plant had normal pollen. Normal pollen would be ca 90% plump, stained, and uniform. Among the plants, 13/28 had pollen of variable sizes. 7/28 had n and 2n pollen. 6/28 had n, 2n, and 4n pollen. Plant 5 had the most 4n pollen, and was reported on the MGR web site in 2006. Plant M8 (now clone M8) was

functionally male sterile, and remaining plants had stained pollen of varying amounts up to about 50% of normal.

Multifoliolate leaves were recorded on eight plants. One plant had a remarkable number of multifoliolate leaves, in that almost every leaf was multifoliolate. There is a message here, because all the parents had normal trifoliolate leaves.

Female fertility as measured by seeds per pod was 0.75 – 3.80 with a mean of 2.42 in open pollination at the Walnut Street Greenhouse field nursery on campus (now the site of a Power Plant). The mean for seeds per pod (s/p) of some cultivars in the same nursery is as follows: CIBA 2444 4.7 s/p; Columbia 2000 4.50 s/p; Pioneer 5454 4.2 s/p; Vernal 3.7 s/p.

Clone M8 in the above study was 2.9 s/p. In 2010, M8 had 3.4 s/p when crossed by hand in a field nursery. Saranac alfalfa clone 6-4ms (now ca 40 years old) in the adjacent row had 5.0 s/p. Hence, female fertility of M8 is below all the cultivar material in the evaluations.

Summary of Hybrid Breakdown observed in the subspecies hybrid plants.

It is important to keep in mind that the plants were hybrids; there was no intentional inbreeding. Nonetheless, every hybrid plant had fertility issues, and several had abnormal leaves not found in parents. The abnormalities are best explained by hybrid breakdown. The subspecies were more diverse than expected. There was segregation distortion for flower color, plants with multifoliolate leaves, reduced female and male fertility in all plants, and some plants produced abnormal 2n and 4n gametes. Likely the information would have remained buried in the project record books, if clone M8 had not surfaced as the most efficient in wide crosses with M. arborea. Since the wide crosses involve the reproductive system, and since there is a breakdown of normal reproduction, the reproductive breakdown appears to weaken the barrier to interspecific hybridization.

The system could be viewed as a bridge-cross, with subspecies hybrids enhancing the ability to make interspecific crosses. Historically, species were used in bridge crosses, whereas a subspecies hybrid is used in our case. Whatever the system is called, a breakdown of normal reproductive processes appears to enable the wide crosses.

Hybrid Breakdown versus Outbreeding Depression, and how to deal with them.

In the 2005 report, and other reports on this web site until 2010, we discussed abnormalities in terms of outbreeding depression. Now that we know more about the extent of the abnormalities, and about the apparent diversity of the subspecies, we are using the term hybrid breakdown. Allard's use of the term outbreeding depression in

1999 was our first exposure to the term, and Allard expected outbreeding depression when he hybridized barley lines with different genetic complexes, and they underwent segregation. Our Medicago subspecies, are much more diverse than Allard's barley lines; hence, we now are using the term hybrid breakdown to describe the abnormalities in advanced generations of the subspecies hybrids.

It is fair to ask: Is the hybrid breakdown in subspecies hybrids, even greater in the interspecific hybrids with M. arborea? Answer: Yes, especially in male fertility, and unusual plants. This begs the question: Can the material still be used in alfalfa breeding? Answer: Yes. Backcross desired traits into alfalfa to recover the alfalfa genotype plus the desired trait. Or, select material that enhances yield, and use it as the seed parent of a hybrid forage product, thus stopping further breakdown. An early generation hybrid product minimizes hybrid breakdown, whereas advancing generations increases it.