**SEGREGATION DISTORTION (SD) IN ALFALFA,
AND THE NATURE OF THE ALFALFA GENOME**

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This report is an outgrowth of The Alfalfa Genome Conference (TAG) and related literature that has impacted on my thinking about the genome. My goal is to gain the best possible understanding using present knowledge, and to plan additional experiments.

SD has been reported in essentially all the DNA-marker studies (see TAG proceedings on the web, and see Kalo et al 2000 Theor. Appl. Genet. 100:641-657, who report some very large SD values, and cite previous DNA-marker studies). SD was also noted in traditional genetic studies (Barnes and Hanson, 1967, Illustrated Genetic Traits in Alfalfa, Tech Bull. 1370, review the literature and mention some of their studies where SD usually involved a deficiency of nulliplexes.

My epiphany on lethals and the nature of the alfalfa genome came when I re-read and then repeated one of my old studies (CJGC 10:357-361, 1968) on a two-gene albino in the F2 of diploid M. sativa x M. falcata. Tech Bull 1370 also reports such a system, and on green, pale green, and albino two gene systems in the F2 of M. sativa x M. falcata. Albinos are seedling lethals and result in SD of genotypes among mature plants. When I got back into this old literature while also reading all the new information, I realized that each species could have a perfectly normal chlorophyll genetic system (the respective parents in the 1968 study were shown to be normal), but that there could be segregation in hybrid generations if the two systems have diverged due to isolation, evolution and speciation. Divergence could be at the level of the gene or the chromosome. Barnes and I discussed our albinos in terms of genes, but I now can see how albinos and other lethals could be produced by deficiencies resulting from recombination involving diverged chromosome segments. Recently, I observed a ratio of 60 green to one albino which is difficult to explain at the gene level.

The connection between the albinos, and the lethals discussed in the DNA marker studies, is that they both cause segregation distortion. I simply want to learn as much as possible about the mechanisms of segregation distortion, and in doing so learn as much as possible about the nature of the genome.

In my mind various possibilities are ripe for testing. The main mechanisms of SD may well be lethals as has been suggested in most studies, but some SD due to chromosome aberrations is also expected. Hence, we should research it.

In the experiments outlined later, I will not be surprised if several traits that I have studied over the years segregate in new hybrid populations dedicated to this

study. Some of these traits follow.
S Multifoliolate leaves; there is more than one genetic system of control, and I have seen one such system emerge in M. sativa x M. falcata hybrid populations. The first time was in thesis work ca 1963, and the last time was Y2K.

S Multiple cotyledons; these show up often in hybrid pedigrees and this year I noted great expression when I repeated the albino work. Also, Barnes notes an association between albinos and multiple cotyledons in Tech. Bull. 1370. And, most of us have seen an association between multiple cotyledons and multifoliolate leaves. Has anyone published on such an association? Can anyone confirm an association?

S 2n gametes; there are several genetic pathways of control, and my experience indicates more 2n and 4n gametes in hybrid populations than in parents. Let's collect more data and find out for sure.

S Homozygous diverged alleles and small deficiencies could relax the normal species developmental processes in all the above and below cases. I think diverged alleles could be null alleles in some cases.

S Black seeds; we know that black seeds have arisen in our diploid M. sativa x M. falcata hybrid populations a few times. We know that a recessive inhibitor is involved.

S The chlorophyll system has been discussed, and should be useful in future experiments.

S Cream flowers are familiar to all of us and fit the null model. I would not be surprised if M. falcata is null at the P locus and if M. sativa is null at the xanthophyll loci. By the way has anyone seen cream M. coerulea?

S We will watch for anything else, e.g. leaf mutants and other mutants reviewed in Tech. Bull. 1370. It is interesting how many traits reviewed in 1370 were observed in advanced generations of M. sativa x M. falcata.

Experiments: Step 1. Make diploid hybrids of CADL x M. falcata; CADL x M. coerulea; and M. falcata x M. coerulea; include reciprocals because I am very interested in how the mitochondrial genome is interacting. We will use CADL derived from Peruvian and other non dormant types because CADL derived from our northern adapted cultivars has a hybrid genome. Also, make pairwise crosses of the parents within CADL, within M. falcata and within M. coerulea. This will permit tracking traits and lethals, and identify those that are contributed by a parent versus those that arise in hybrid backgrounds. The situation will be parents as controls versus hybrid populations.

Step 2. Use sibbing as well as selfing in parent and hybrid populations so that no parents and no F1s are excluded from analysis because they are self-sterile.

Most of us have used the most self-fertile Fls, however, I now would like to examine a full range of materials.
Step 3. Screen for traits that segregate in the parents and in the hybrid generations. Look at the chromosome pairing in the PMCs of parents and hybrids of materials that segregate for interesting things. Spot check as many hybrids as possible. Want to see a nice heterozygous translocation? See CS 3:147-150.

Step 4. Clone the parents and F1s of particularly interesting pedigrees.

Step 5. Make stocks available for traditional and DNA research. I see a great potential for identifying "naked-eye polymorphisms" (NEPS; credit Ed Coe for this term) and then mapping and learning more about them at the DNA level. The potential for research at the DNA level is almost limitless, not just in these stocks but all materials. For example, I hope someone will look at SD in original and improved materials. Is the magnitude of SD inversely associated with improvement?

Concluding Remarks

S SD is a fact of life in alfalfa reproduction.

S SD varies from region to region on each chromosome.

S The chromosome blocks (units of genetic transmission) are not all transmitted equally and one set of assumptions does not apply.

S Theoretically, quantitative genetic analysis is thrown into a "tailspin" in many mating strategies, especially where gametic lethals are involved.

S Disomic segregation in diploids and,

S Tetrasomic segregation in tetraploids are modified from region to region by SD.

S The mechanisms that can cause segregation distortion are generally known, and include lethals, chromosome aberrations, and essentially all other forms of mutation including transposable elements.

S Lessons from somaclonal variation research suggest that all mechanisms probably are involved, but lethals and chromosome aberrations top my list, in terms of frequency and importance.

S What is the nature of the lethal genes? Why are there so many? The dedicated new hybrid generations may help inform us.

S The potential of genetic erosion of favorable alleles linked to lethals haunts me! I worry about inbreeding by selfing!

S Then, I read Allard's 1999 paper in Annual Review of Genetics 33:1-27 and I realize I must keep selfing. However, henceforth we need to do at least a few tight experiments to test the magnitude of such erosion, if it exists. Ian Ray wrestled with this problem (CS 32:336-339) and suggests a great experiment in the last paragraph.

S My current thinking about the genome essentially intensifies the importance of maximum heterozygosity involving multiple allelles or favorable dominants in linkage blocks (CS 34:823-829).

S Moreover, I believe it intensifies the need to identify (or build) heterotic groups as per Brummer, CS 39:943-954.

S The heavy genetic load of lethals, null alleles, etc. etc. is tolerated because of random bivalent pairing and resulting tetrasomic segregation.

S The SD findings help explain the severe inbreeding depression in alfalfa.