**Notes on Segregation Distortion, Novel Traits, and Transgressive Segregation 1902 – 2002
While Thinking About the Alfalfa Genome1**

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Prologue: This report is a work in progress that has changed my thinking about the alfalfa genome, its relationship to the genomes of the crossable perennial species, and about speciation in *Medicago*. Until I reviewed the literature cited in this report, I was frustrated about many things that I did not understand last century. Now, everything seems to be falling into place. The alfalfa genome is beginning to yield its secrets! Moreover, some new experiments using existing stocks to test new hypotheses, should put capstones on some important genomic relationships.

Introduction

Segregation distortion (SD) is a common feature of papers on mapping alfalfa using molecular markers. This will be discussed in relation to the genome after considering the general area of segregation distortion in plants. Over the years, SD has been termed selective fertilization, unequal segregation, altered, irregular, and blurred segregation. It is discussed in terms of meiotic drive and even outlaw genes: and, novel variation in some interspecific hybrids is often associated with SD. I started reading the most recent literature, and worked back in time. This was a fascinating, but unsettling experience as I discovered and rediscovered literature that I had missed over the years or that I had not considered relevant to my alfalfa breeding and genetics research.

Early Reports of Segregation Distortion

These notes will begin with the earliest reports of SD, which so happen to coincide with the rediscovery of Mendelian genetics. A wealth of information was found in a delightful little book by Donald F. Jones entitled "Selective Fertilization" (1928). I thank Jerry Kermicle for calling the book to my attention.

Jones (1928) pointed out that in the first detailed investigations of heredity following Mendel, Correns found that certain crosses of maize did not have the expected proportion of one-fourth recessives. Correns reported this in 1902 based on results from a cross of pointed popcorn with a sweet, wrinkled variety. In a large number of trials the sugary factor pair (Susu) usually segregated for 25% recessives except for one situation where Correns observed 16% recessives. This deficiency of recessives was confirmed by Jones and by other prominent researchers including Lock (1906) and East and Hayes (1911) (see Jones 1928 for refs). Not only did Jones confirm Correns, he also observed exactly 16% recessives based on 3,681 seeds classified for sugary. In the full study, Jones compared five situations involving combinations of heterozygous and homozygous sugary, and found that there was no deficiency in the percentage of recessives, i.e. no differential fertilization in those matings in which the male gametes were all alike. A selective action took place only when segregating pollen was used. Hence, the selective fertilization took place only in certain cross combinations and only in segregating pollen.

Jones reviewed other examples of deficiency of recessives in maize, *Melandrium*, *Lychnis*, *Rumex*, *Datura*, and *Oenothera*. Jones' general conclusion was that the transmission of heritable characters is dependent on many things:

- The position of the genes in the chromosomes.
- The usual and unusual assortment of chromosomes.
- The elimination of gametes due to lethal factors.
- The elimination of zygotes.
- The union of particular gametes in greater numbers than expected.

Jones then went on to review and discuss the last point in terms of differences in the rate of pollen tube growth. His conclusion was that most of the deviations from expected ratios in his work were due to pollen tube factors.

The area of gametophytic factors in maize that affect fertilization is extensive and was reviewed by Oliver Nelson in 1994. Nelson reviews much research by Mangelsdorf, Jones, Emerson, Demerec, Schwartz and himself (see Nelson for refs.). Kermicle and Allen (1990) reported an extensive study of cross incompatibility between maize and teosinte, and discussed how the Ga1-s system could function as a barrier to pollination. Recently, Kermicle has proposed utilizing the strategy to prevent genetically modified maize from crossing with normal maize.   Thus, selective fertilization is just as relevant today as when Jones published his book in 1928. The SD in maize is controlled by genes already in place in the maize genome. In much of the review that follows, there is both this type of SD, but also SD that results from crossing over involving chromosomes that have various degrees of divergence.

Segregation of Novel Traits

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An early study by Lotsy (1915, 1916) was cited by Stebbins (1950), Rick and Smith (1953) and Grant (1975) (all of whom will be reviewed later). Lotsy found new traits that segregated in the F2 of a hybrid of _Antirrhinum glutinosum_ and _A. majus_. The Lotsy references are available in the U.W. libraries. Both _Antirrhinum_ species have the typical flower with a prominent two-lipped corolla limb. Some individuals in the F2 generation of the interspecific cross had tubular corollas with reduced limbs, as in the related genus _Rhinanthus_. This unusual flower type occurred sporadically in the F2 and Lotsy stabilized it in some F4 lines. Lotsy was confident that this was new variation and in fact wrote a book entitled: "Evolution by Means of Hybridization" (1916). His study remains controversial, however, because he did not provide evidence that the mutation was not carried by one of the parents.

Another study involving different strains of the same two species went to great lengths to show that a new variant had arisen in the F2 of the species hybrid, and was not hidden by heterozygosity in one of the parents. This research was by Kenneth Mather at Birmingham, England.   His initial study involved making the species crosses between _A. majus_ and _A. glutinosum_ (Mather, 1947). Mather could find no mechanism isolating the two species once pollen had been successfully transferred to a stigma of the opposite type. Pollination by artificial means was easy between these species as within them. Furthermore, when stigmata of either species were pollinated simultaneously with pollen from the two, hybrids were produced as commonly as maternal types. (The discussion could just as well be about _Medicago sativa_ and _M. falcata_.) Mather's observations of bee behavior brought him to the conclusion that the mechanism isolating _A. majus_ and _A. glutinosum_ was to be found in bee preference for one type or the other. (Does this remind you of alfalfa, or what?)

In a follow-up to the original _A. majus_ by _A. glutinosum_ hybrids, Mather and Vines (1951) report discovering cleistogamy in derivatives of the hybrid. They studied large sample sizes of parent and hybrid populations, used statistics appropriate for Mather, and concluded that cleistogamy had not been seen in the parental species nor in the F1 generation. They suggested that 2 -3 genes of supplementary (complementary) effect are necessary for cleistogamous flowers. They do not attempt to suggest a mechanism for the origin of the novel variation; but, Grant 1975 suggests that the new variation seen by Mather and Vines represents "macrorecombination". Mather and Vines state: "Thus the two parental species, one an obligatory cross-breeder and the other regularly setting a considerable fraction of its seed by cross-pollination, contain between them all the genetical materials necessary for the production of an inbreeding type of plant, i.e. a type of a plant with a breeding system distinct from, and even opposed to those of its parents."

Stebbins (1950) has a section on page 279 that discusses hybridization and the origin of new types. Stebbins discusses how hybridization of species mainly produces convergence between previously distinct species. He goes on to point out that there is evidence that in some instances hybridization can result in the appearance of types which are actually new. The review of this evidence was thrilling to me. The original references were consulted whenever possible. Stebbins writes on page 285, "Some evidence at hand suggests that the recombination of genetic factors in the offspring of interspecific hybrids may sometimes lead to new types radically different from those found in either parent." He reviews Lotsy, 1915, indicating that the paper contains some striking examples of new traits in the progeny of _Antirrhinum glutinosum_ crossed with a peloric form of _A. majus_. From Stebbins, I learned about Hagedoorn and Hagedoorn (1921) who cite the example of Vilmorin's hybrid between _Argemone mexicana_ and _A. platyceras_, from which several strongly aberrant types segregated in the F_2 generation.

Some of these were different from either parent in such fundamental characteristics as the number of sepals or of carpels. Similar aberrant types appeared in the F₁ hybrids of *Paeonia lactiflora* (*"P. albiflora"*) and various members of the complex of *P. anomala* (Saunders and Stebbins 1938). Less extreme new types are reported by Clausen (1926) as segregates from hybrids between *Viola arvensis* and *V. tricolor*.

At this point I realized that we had not understood the significance of the black seed trait that segregated in backcross generations of our haploid X *M. falcata* materials. The black seed trait is certainly novel because it does not occur in either *M. sativa* or *M. falcata*. It is complex in inheritance (Kimbeng and Bingham 1997) which is evidence of variation resulting from recombination, in my view, currently. To our knowledge, the only other *Medicago* species where black seeds occur is *M. intertexta*. There is some kind of an evolutionary statement being made in the segregation of black seeds in advanced hybrid generations of *M. sativa* and *falcata*. It is my recollection that the black seed Don Barnes worked with goes back to an old *M. varia* population, which descended from a hybrid of *M. sativa and M. falcata*.

Now, we turn to another book that was a rich source of references on novel traits: Grant (1975) Genetics of Flowering Plants. In his chapter on gene interaction, Grant has a section titled: Macrorecombinations. Grant defines macrorecombinations as: "Morphological characteristics unlike those found in either parent," and indicates that he coined the term (Grant 1956. He indicates that macrorecombinations occasionally appear in the progeny of interspecific hybrids.. Grant further states that, the phenomenon has long been known, and starts his review with Lotsy (1916). Lotsy 1916 is a follow-up to the 1915 paper reviewed earlier in this section.

Grant (1956) also reported on his work with *Gilia*, involving a case where new variation emerged in the F3 generation of a cross between *G. achilleaefolia* and *G. millefoliata*. The variants had altered flower morphology and variant frequency was greater in F4 families derived from single-plant selections. Other examples of what Grant terms macrorecombinations can be found in advanced generations of interspecific hybrids of *Gossypium, Geum, Lycopersicon* and *Layia*, as reviewed by Grant (1975).

Variants in *Lycopersicon* were reported by Rick and Smith (1953) in a paper titled "Novel Variation in Tomato Species Hybrids". This paper was pivotal in my thinking about variation that I have seen in advanced hybrid generations of *Medicago sativa* and *M. falcata* at both diploid and tetraploid levels. The crosses used *L. esculentum* as the female, *L. peruvianum* as the male, and involved different parents of each species in two different hybrids. *L. esculentum* is the familiar tomato and *peruvianum* has small green fruits, several forms of resistance, and is high in vitamin C.

Rick and Smith's Hybrid No. 1, as they referred to it in the paper, was between *L. esculentum* var. Michigan State "Forcing" and *L. peruvianum* var. dentatum PI128657. It was a classic interspecific hybrid in that the hybrid was difficult to make and it was difficult to obtain the F2 by selfing. The first two variants to be discussed appeared among 46 F2 plants. The authors discussed the fact that the F2 segregated for such a wide array of recombinations of parental characters that no two individuals were alike. What they considered variants were entirely outside the normal range of segregation. The first variant was termed "*entire leaf*"; other features accompanying the leaf modification were reduction in vigor and slight asymmetry of the flower. (The description makes Bingham think of the *cauliflower head-simple leaf trait* in alfalfa, also known currently as *uni*, Brouwer and Osborn, 1996).

The second variant in Hybrid No. 1 was "*compound inflorescence*". Inflorescences are so greatly subdivided that each one bears several hundred flowers. This compares to tomato with 3-9 flowers, *L. peruvianum* with 15-40, and the F1 hybrid with 8-20 flowers. The authors point out that proliferations of flowers to this degree were never seen in any of their lines, and it is certain that this gene was not present in the *L. esculentum*. The *L. peruvianum* parent was self-incompatible and could not be tested for either trait, although it was apparent that the authors had not previously seen either of the variant traits in their lines.

Another novel variant with a coppery or tawny color in the corolla, also had not been seen before by the authors. It occurred in the F2 of a hybrid involving a different tomato variety and an accession of *L. chilense*. The variant was termed "old-gold corolla". The basic recessive gene for old-gold was found to exist in the *L. chilense* parent, but the exact phenotype in the hybrid required new interactions involving the tomato parent. The authors indicated that"...it is conceivable that complementary gene action of some sort between old-gold and genes from *L. esculentum* might be necessary for the observed phenotypic expression." Hence, it was considered novel variation.

Rick and Smith (1953) cite four other novel variants they recovered from interspecific crosses: 1) dark pigmentation of anther tubes, 2) dialytic anthers, 3) chlorophyll-deficiency and 4) sticky-chromosome-sterility trait. The authors discuss potential mechanisms that could produce novel variation in species hybrids. The mechanisms are listed below without discussion to save space at this point.

Mutation. The possibility of increased mutability in species hybrids.
Complementary action of genes of the parent species. This will be discussed again later.
Recessive genes derived from the self-incompatible parent. Recessive traits seldom or never seen
       because of enforced out crossing.
Additional mechanisms that might be mentioned currently include:
Activation of transposable elements.
Altered patterns of methylation.
Cytogenetic divergence. Translocations, inversions and/or what Stebbins (1950) and Stephens (1950)
       term "cryptic structural difference".
Notice that some of these mechanisms also could produce SD.

The Transition to SD in Rick's Tomato Research

The following reference is reviewed more thoroughly than usual for several reasons. First, it is an excellent example of SD in interspecific crosses. Second, it carefully separates SD due to presyngamic and postsyngamic causes. Third, it is the basic model for our ongoing analysis of hybrids of *M. sativa, coerulea* and *falcata*.

Rick (1963) published an in depth analysis of SD in a tomato species hybrid of *L. esculentum* and *L. chilense.* He pointed out that species hybrids often are associated with "departures from normal segregation". Causes cited by Rick include: 1. structural differences in chromosomes which can lead to a wide array of cytogenetic abnormalities and unequal segregation, 2. meiotic drive, 3. lethality of gametes, and 4. lethality in postsyngamic stages. Evolution in the thinking about SD can be seen in the lists of possible mechanisms. Ten years after Rick and Smith (1953), Rick (1963) does not cite mutation and includes chromosome divergence at the top of the list.

The genus *Lycopersicon* according to Rick is ideal for such investigations because its species can be hybridized in many combinations, the F1s are reasonably fertile, monogenic markers are available, and the meiosis of F1 hybrids is not complicated by differences in number or gross structure of chromosomes. Rick further reviewed the fact that genetic segregations are generally (but not always) normal in crosses between closely related species, but become more disturbed in wider crosses. Thus, the degree to which genetic segregations are disturbed is a measure of genetic relatedness.

Three *L. esculentum* parents were used that among them brought seven recessive and two dominant traits into the analysis. Two lines of *L. chileuse* from Peru carried the wild type alleles. The F1 hybrids were phenotypically normal in all combinations with the markers indicating that the *L. chilense* parent contributed the normal alleles in their respective hybrids. The F1 hybrids could only be obtained using *L. esculentum* as the seed parent, and by embryo culture. Once F1s are produced, the F2 and backcross generations require no special treatments except to make sufficiently large numbers of crosses. Chromosome pairing in hybrids always appeared complete in prophase of the first meiotic division, and no major abnormalities were observed at pachytene or later stages. Nonetheless, approximately one-half of the pollen was aborted in the F1 hybrids.

For each of the recessive genes tested in the F2, the yield of homozygous mutants was below expectation and highly significant. The SD was greatest for lutescent foliage, where there were half as many mutants as expected. This meant that there was greater distortion toward the wild-type parent in the F2.

The backcrosses to *L. esculentum* showed SD toward an excess of respective *esculentum* alleles, although the differences were significant for only dwarf and lutescent. The gene for lutescent had the most SD in the F2 and the backcross, and the next highest was dwarf. Location of the genes on the chromosomes is known in tomato, but Rick pointed out that he could not determine whether the SD involved a chromosome or a chromosome segment. To do this would have required more markers on respective chromosomes.

Rick discussed the fact that data based on qualitative traits and therefore monogenic segregations were the most precise measures of SD, but went on to make some interesting statements about SD involving quantitative characters. According to Rick the first hints of SD in interspecific hybrids in his program were detected in quantitative traits, although he does not indicate which traits. It logically follows that if monogenic segregations are disturbed, so will be the segregation of quantitative characters that are determined by a group of independent genes.

Now comes the critical determination of whether the SD is due to presyngamic or postsyngamic causes. The list of genetic and chromosomal mechanisms at the beginning of this section should result in

unequal F1 gamete ratios that should deviate in the same direction regardless of the other parent in the cross. This is the key. But, in contrast, Rick's data revealed deviations toward an excess of *esculentum* alleles in the backcross to this species, and toward an excess of *chileuse* alleles in the F1 and backcross to that species. Hence, the causes of SD in Rick's study are postsyngamic.

The genus *Melilotus* is considered by many to be a close relative of *Medicago*. Baenziger and Greenshields (1957) reported that irregular ratios were common in derivatives of interspecific crosses of *Melilotus alba* x *M.dentata*, and *M. officinalis* x *M. alba*. Previous studies using pure *M. alba* reported simple Mendelian inheritance for coumarin and permeable seed coats. However, in their interspecific materials some plants produced F2 segregations that did not fit any Mendelian ratio. They used the term "blurred ratios" in addition to irregular ratios; SD seems to inspire interesting metaphors. The SD in their data varied from slight and not significant, to more than 50% deviation that was significant. They discussed their SD in terms of structural differences between the respective genomes that could give rise to the irregular segregations. Moreover, they state: "It appears that irregular segregation is an effect associated with interspecific hybridization". Oh how I wish that I had internalized this relationship, and realized early in my career that it could possibly apply to hybrids of *M. sativa* and *M. falcata*. Oh well, better late than never.

       In 1927, R. A. Brink, the geneticist involved in the development of Vernal alfalfa, published and interesting paper titled: Whence Come the Rogues in Canning Peas? The title was essentially a metaphor for the origin of novel variation in general. Brink discussed the phenomenon in terms of mutation of genes, rather than chromosome divergence and unequal crossing over. I believe that both mechanisms are involved broadly, but conclude from all that I have read that the mechanism with the greatest impact involves chromosomes and recombination.
Recall that the transition in Ricks interpretation were also in this direction.

Important Writings of S. G. Stephens, Cotton Cytogeneticist

       Stephens' (1950, 1951) research on interspecific hybrids in cotton is well known and widely cited. Stephens may have been the first to use "blurred ratios" for SD. He found blurred monofactorial ratios and skewed backcross ratios in cotton, and ascribed these to structural differences between the genomes involved in the interspecific crosses. He further noted that these structural differences are too small to be detected cytologically and could be described as "cryptic structural differences". In a latter paper, Stephens 1961, he credits Stebbins for originating the term cryptic structural differences. This 1961 paper by Stephens, cotton geneticist at North Carolina State University, Raleigh, NC, deserves special attention in my view. It begins on page one of volume one of Crop Science. I have read it more than once over the years, but it now has its greatest impact on me. Stephens was ahead of his time in pointing out that polyploidy is a continuous evolutionary system based on levels of genomic divergence. Stephens did not discuss polyploids in terms of allo-or autopolyploids, or even disomic or polysomic polyploids, but placed them in four essentially overlapping groups within a continuous evolutionary system.
Moreover, Stephens'concept of a continuous evolutionary system illustrates the continuous and overlapping nature of SD, novel traits, and transgressive segregation, in my view.

Group I. Parental genomes are cytogenetically homologous in the sense that their chromosomes pair regularly and recombine without apparent reduction in fertility. Stephen's examples: The cultivated species of *Hordeum*, where he cites L. Smith, 1951, Bot. Rev.17: 1-51; and the genome of common tetraploid and hexaploid species of *Triticum*, where he cites E. Sears, 1948, Adv. In Genetics 2:239-270. As Sears and others have pointed out, wheat in the absence of genetic control of chromosome pairing specificity would behave as an autopolyploid.

Group II. Parental genomes pair regularly or almost regularly at the diploid level and show no evidence of preferential pairing in the tetraploid derivative. [This describes the *M. sativa-coerulea-falcata* complex] **Recombination in later generations is accompanied by reduction in fertility, departures from expected Mendelian ratios and a range of variation that extends beyond the ranges of the parental species.** His examples are from his own work in *Gossypium*, and in *Lycopersicon*, including the work of Rick that we have reviewed. I now believe that the intercrossing *Medicago sativa-coerulea-falcata* complex falls in this group. I will be reevaluating my old work, and

continuing to gather new data on this for the rest of my life. In my view, this is the source of SD, of segregation of novel traits, of many forms of pest and stress resistance, multifoliolate leaves, 2n gametes, black seeds, and so forth; and it is where transgressive segregation and outbreeding depression are coming from. Once again, I will be collecting data on this the rest of my life, but everything is falling into place, and I now believe that divergence in the respective genomes and recombination produce much of the above variation, and that it is the mother load of genetic load in Medicago. And, it appears to me that the variation can arise in intra-or interspecific cross populations. The literature of the last century indicates populations that have been reproductively isolated for a period of time have the potential for divergence and variation produced by recombination.

Group III. Parental genomes pair regularly or almost regularly at the diploid level with marked preferential pairing at the tetraploid level. Recombination in later generations is associated with phenomena similar to those characteristics of group II. His examples are in *Gossypium* and *Solanum*. As of 2003, I believe that we could have some preferential pairing in the crossable *M. sativa-coerulea-falcata* complex, but that we have not found it because we tend to cross our hybrid species derivatives, and crossing breaks up potential preferential pairing. Hence, we are now examining our hybrid derivatives by selfing.

Group IV. The parental genomes show partial or complete failure to pair in their hybrid. Transmission of unpaired chromosomes to backcross progenies and their derivatives gives rise to trisomics, substitution and alien addition races, and various unbalanced combinations. Typically, the species that fall into groups I and II based on cytogenetic behavior have been termed autopolyploids and those in groups III and IV termed allopolyploids. The advantage of Stephens classification is that it keeps me thinking about a continuous evolutionary system with its emphasis on increasing divergence and the consequences of recombination. Essentially, my whole career is passing before my eyes at this point, and I see things differently. Many things that I could not explain now have an explanation. Rather than being frustrating, the situation is generating peace of mind about the alfalfa genome, about inbreeding depression, about complementing chromosome blocks and how they breakdown in advanced generations.

Zamir and Tadmor 1986; Another Important Paper

A paper by Zamir and Tadmor (1986) was very important in my thinking about the alfalfa genome. I thank Michael Havey for bringing it to my attention. Zamir and Tadmor use the term unequal segregation for segregation ratios that deviate from expected. They examined monogenic segregation data from their published and unpublished experiments, and from other literature for 17 crosses involving different evolutionary relatedness in the genera *Lens, Capsicum,* and *Lycopersicon*. Morphological markers (the naked-eye polymorphisms of Ed Coe) were employed principally for intraspecific comparisons since, within species, enzymatic genes are often monomorphic. This helped me realize the importance of the few good morphological markers that could be tracked in alfalfa. Segregation in interspecific crosses is studied mainly using alleles determining electrophoretically variant enzymes, that are usually co-dominant and thus have the advantage that alternate alleles do not affect plant survival. I thought about the marker problem in terms of our alfalfa research, and I am using the same two morphological markers in both intra- and interspecific crosses. These are cream flower color (p) and the basic anthocyanin factor (c2). There are a few more potential traditional markers, but they are not in place, and I do not have time to backcross them. It seems to me that the perfect study would use the same markers in both intra- and inter-specific crosses.

Zamir and Tadmor (1986) found SD in both intra- and interspecific crosses in *Lens, Capsicum,* and *Lycopersicon*, but the magnitude was greater in interspecific crosses in all cases. Overall, the proportion of loci deviating from the expected monogenic segregation ratios in interspecific crosses was significantly higher (61/114 genes, 54%), than in intra-specific crosses (7/52 genes, 13%). Zamir and Tadmor may have been the first to state: "such unequal segregations should be considered in planning breeding programs". Zamir and Tadmor were the authors who lead me to Dawkins (1982) who discussed segregation distorters as "outlaw genes". I consider this one of the most colorful metaphors in genetics. Dawkins, however, credits Alexander and Borgia for the term outlaw genes (ref. in Dawkins).

Recent Research on the Origin of Novel Traits and Genome Change

A frequently cited paper by Song et al. from Tom Osborns lab in 1995 essentially initiated the current interest in polyploid genomes. They report rapid genome change in newly synthesized allopolyploids of *Brassica*. Most genome changes involved loss and/or gain of parental restriction fragments and appearance of novel fragments. Chromosome rearrangements resulting from intergenomic recombination involving homoeologous chromosomes were thought to be a major factor. This study inspired a great deal of interest in the dynamics of polyploid genomes and the segregation of novel traits.   See Osborn, et al. 2003 for literature.

Genome change in a newly synthesized allopolyploid in *Medicago* also has been reported by McCoy et al. 1991. They report loss and/or gain of parental markers in the hybrid of *M. sativa* X *M. papillosa*. They examined 20 isozyme and RFLP markers segregating in the F1. Segregations were essentially disomic indicative of allotetraploid behavior of the hybrid, but there were exceptions. Exceptions were where progeny failed to receive an allele or received an extra allele. Of the several possible mechanisms that could cause the exceptions, the most likely cause was a low frequency of homoeologous chromosome pairing and recombination between the diverged chromosomes of *M. sativa* and *M. papillosa*. It would have been great if this paper could have been cited in the Song et al. paper in 1995; I believe that it demonstrates the near universality of the phenomenon.

These types of abnormal segregations in *Brassica* and *Medicago* represent the allopolyploid end of a continuous system of chromosome divergence as presented by Stephens 1961. Moreover, it helps me understand the SD in the *Medicago* mapping exercises, as well as the segregation of albinos, 2n gametes, lethals, and other abnormal segregations in our Wisconsin material.

Selected Literature and Attempts At Unifying Knowledge About The Medicago Genome

The literature begins to expand about this time, and I will have to be more selective from here on. An excellent recent paper is a genome-wide survey of reproductive barriers in a hybrid of *Japonica* X *Indica* rice (Harushima et al. 2002). They identified 33 reproductive barriers, 15 affecting the gametophyte, 18 altering viability of the zygote, and reported a very interesting case of almost exclusive transmission (94%) of an *Indica* allele through the male gametophyte.

There is a lot of SD in the alfalfa mapping papers, and all used *Medicago* *sativa* X *M*. *falcata, or M. coerulea* X *M. falcata* (see literature cited in Kalo et al. 2001).   Recall that until recently, *M. coerulea* was known as diploid *M. sativa*. SD also has been found in alfalfa materials with less apparent diversity (Brouwer and Osborn 1997a, 1997b). SD in Medicago needs a comprehensive review. Who would like to do it?   The next section contains my current thinking about the genomic situation in the crossable perennial complex. Last year on this website under the title of SD and the Nature of the Alfalfa Genome, I discussed how I have reevaluated a 1968 study of mine on an albino that segregated as a two gene trait in an F2 of *M. sativa* (now *coerulea*) X *M. falcata*. The SD reported in alfalfa caused me to think about the potential divergence between the genomes involved, and how recombination involving diverged chromosomes could result in deficiencies that could cause albinos. I have since repeated the study with new materials and have found another case where the F2 segregates for an albino when neither parent segregated. It was one cross combination out of six that were analyzed, nonetheless, it did not take long to repeat it. In this case the albino segregation is ca 35:one, i.e.,a non-Mendelian segregation for a diploid. Also in the last year, crosses involving *M. truncatula* cv. Jemalong crossed with cv. Caliph, and with cv. Paraggio, segregated for albinos in non-Mendelian ratios that were different in each cross. (See this website, Vol. 2.) And, Thierry Huguet, in personal communication has indicated that Jemalong by one of his accessions also segregated for an albino. In fact, Huguet had a two gene model worked out for it, just like Barnes did, and Bingham did, in hybrid populations of M. sativa X *falcata* in the late 1960s. Read on and see how Lynch's model involving chromosome divergence and gaps could explain it.   Back to *M. truncatula*, where a total of three different pedigrees segregated for albinos and all involve Jemalong. This suggests to me that there is some kind of chromosome divergence in Jemalong, perhaps a homozygous translocation.   Also in our *M. truncatula* crosses, I observed segregation for the same types of things that I have observed all of my career in *M. sativa* X *M. falcata* derivatives at both diploid and tetraploid levels. These include multifoliolate leaves, extra cotyledons, lethals, fertility differences, differences in hard seed content, SD for some traits,etc.. In fact, I have a very unified feeling about the *Medicagos* after working with various species which now includes *M. lupulina*, and M*. arborea* in addition to those mentioned above.

Back to the literature

In a paper by Michael Lynch in Science in 2002, the figure could just as well be labeled *M. sativa and M. falcata* in terms of an ancestral species (unknown in our case) acquiring duplicate genes, probably by unequal crossing over, and then diverging due to geographical isolation. Then in isolation, there is gene silencing of some duplicated genes, and there could be more types of divergence in isolation. Now when the two species are hybridized as has frequently been done in *M. sativa* and *M. falcata*, recombination will give rise to null alleles (lethals, sublethals, etc.) that will result in nonfunctional gametes and nonfunctional progeny; essentially everything that we have been discussing. Importantly, the same types of gametes also are produced by recombination involving macro- or microchromosomal divergence. And, in the crossable *Medicago* perennials, there are several reports of karyotype differences, differences in heterochromatin, coiling, length, etc; hence, I am going to start reviewing the papers by Stanford, Clement, Lesins, Gillies and Lesins, Gillies, Gillies and Bingham, Simon and Simon, Bauchan, and others.

The literature search for knowledge is over (for the time being) with the publication of a paper in PNAS by Fu and Dooner, 2002, on how unequal crossing over at the level of the allele can lead to lethal deletions, and genetic load. In fact, Fu and Dooner even mention that their results in maize may apply to other species that show strong inbreeding depression like alfalfa. I agree completely. But, I think that variation generated by recombination errors at the allele level will naturally be less than that generated at the chromosome level, especially when there is chromosomal divergence.   Now, when we summarize where divergence and unequal crossing over can occur, and we consider the geographical and reproductive isolation involved in perennial *Medicago* species and even accessions within species, it is no wonder that we have SD, novel traits, transgressive segrgation, outbreeding depression, inbreeding depression, as well as heterosis when we cover or complement the deficiencies. In fact, I think that divergence and the associated meiotic consequences explain much of the genetic load in perennial *Medicagos.*

It is easy for me to see how we can use the same materials that we used last century in new experiments to test all of the above mechanisms. However, I am especially interested in outbreeding depression. I am dropping several projects in order to do an experiment on outbreeding depression where no inbreeding is involved. Inbreeding and inbreeding depression were used as the excuse for many problems last century, and I want them out of this equation.   If we reject the null hypothesis and have to accept the fact that outbreeding depression is a fact of life in alfalfa breeding, then we will be able to make changes in breeding strategies to cope with it this century. Allard 1999, in his paper on the history of population genetics in plants, introduced me to the concept. Outbreeding depression in plants and animals also was reviewed by Lynch and Walsh 1998. It can occur when parents that have been reproductively isolated are crossed. Heterosis can be quite good, but progeny in advanced generations can be less fit than members of the original parental lines, due to the breakup of adaptive complexes. This also can be exaggerated by recombination involving divergent chromosome complexes, as we have been discussing. One thing is for sure, synthetics enable outbreeding depression, whereas hybrids prevent it.

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