AN ILLUSTRATED SUMMARY OF GENETIC TRAITS IN TETRAPLOID AND DIPLOID ALFALFA

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AN ILLUSTRATED SUMMARY OF GENETIC TRAITS IN TETRAPLOID AND DIPLOID ALFALFA

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INTRODUCTION

Simply inherited markers in alfalfa are urgently needed for research on genetics, breeding, and seed production, especially for studies dealing with heterosis, genetic shifts, and habits of pollinators. This publication compiles published and unpublished information on genetic markers studied in the *Medicago sativa* L. species complex in a summary form and makes such information more accessible. The compilation is limited largely to traits for which a factorial hypothesis of inheritance has been proposed. Some of the traits are more useful as markers than others.

Information presented on each trait consists principally of a description and a brief account of its mode of inheritance. Whenever possible, black and white photographs are used for illustration. Inasmuch as some expressions are best shown in color, the number that can be effectively illustrated here is limited.

Genetic symbols and nomenclature are in accordance with usage recommended by the Alfalfa Improvement Conference. In some instances symbols were changed, but only with the concurrence of the originating author.

TETRAPLOID ALFALFA (2N = 32) Hypocotyl and Root Traits

Elongated Hypocotyl

Davis $(21)^1$ reported that elongated hypocotyl (fig. 1) was conditioned by a recessive gene inherited in a tetrasomic manner. He noted a deficiency in the recessive class, which he attributed to differential gametic viability. He proposed the gene symbol *el*. The original mutant was found in an S₁ family and was partly chlorophyll deficient. However, it had sufficient chlorophyll to produce a normal-sized mature plant. Chlorophyll deficiency and elongated hypocotyl appeared to be conditioned by the same recessive gene in the nulliplex condition, but a hypothesis of closely linked genes could not be ruled out by the author.

¹ Italic numbers in parentheses refer to References, p.37. References to unpublished master's and doctors' theses have also been included.



FIGURE 1.--*A*, Seedlings with elongated hypocotyls; *B*, seedlings with hypocotyls of normal length. (Courtesy Agronomy Department, Purdue University.)

Red Root

Red root, caused by a red pigment in the root cortex, is not clearly differentiated until seedlings are 5 to 6 weeks old. Younger seedlings can be accurately classified if the red pigment is intensified by dipping roots into concentrated hydrochloric acid. The occurrence of red root mutants in alfalfa was known nearly 20 years before the inheritance of the trait was studied by Stanford.² He concluded that red root was conditioned by a single dominant gene Rd with tetrasomic inheritance. He found no indication of either zygotic or gametic inviability associated with the red root allele. Barnes and Cleveland (4), however, observed a deficiency of red root segregates when the Rd allele was transmitted through the pollen. Transmission through the seed parent appeared normal.

Rough Root

A heritable rough root trait, superficially similar to a condition arising from winter injury, was described by Jones (27). The roughness originated from an abnormal periderm. The phellogen of rough root plants developed deeper in the root cortex than in normal plants, and remained continuously active, but the thickened phellem produced did not appear to protect underlying cells. Subsequently, a new cambial activity was incited, which usually failed. Most plants with this trait did not transplant well. In the study described by Jones, all S_1 progenies from rough root

²STANFORD, E.H. October 30, 1961. [Personal correspondence.]

3

plants had abnormal roots, but the degree of roughness often varied among plants. All plants with rough roots, however, could be identified. Crosses between rough root and normal plants produced F_1 plants with normal roots. From 2 to 20 percent of the individuals in three F_2 populations of about 50 plants had rough roots. A genetic hypothesis was not presented.

Leaf and Stem Traits

Crinkled Leaf

Odland and Lepper (37) described a plant with crinkled leaves, which was observed in the progeny of a cross between tetraploid *Medicago sativa* and tetraploid *M. falcata* L. They ascribed the abnormality to the epidermis and mesophyll growing at a more rapid rate than the vascular tissue. The degree of leaf crinkling varied among plants. A large amount of crinkling was detrimental to the plant and resulted in stunting and restricted flower production. On the basis of F_2 and limited F_3 data, they concluded that two complementary dominant factors *D* and *E* were present in normal plants. Absence of either of the two dominant factors produced the crinkled trait. A third factor, dominant *Cr*, also produced crinkled plants, but only when factors D and E were recessive. Disomic inheritance was proposed for the three factors, but the possibility of tetrasomic inheritance was not investigated.

A phenotypically similar trait (fig. 2) was studied in advanced generations of a cross between diploid *M. sativa* and diploid *M. falcata*. (See Crinkled Leaf, p.30.)

Dwarfness

Pauli and Sorensen (42) described a dwarf mutant characterized by greatly shortened internodes and a dense rosette of small leaves close to the ground (fig. 3). Dwarf plants failed to develop floral primordia under conditions that resulted in profuse flowering of normal plants. Flowering and seed set developed only after applications of gibberellic acid to soil in which the dwarfs were growing. Sorensen³ found that dwarf ness was conditioned by a recessive gene dw and expressed in the nulliplex condition. He postulated a tetrasomic mode of inheritance.

Busbice (13) reported finding two additional dwarf mutants with phenotypes similar to the dw dwarf described by Pauli and Sorensen. Dwarfness in each case was controlled by a single recessive gene with tetrasomic inheritance. Intercrosses between the two dwarf mutants produced normal F₁ progenies. Busbice, therefore, concluded that they were controlled by different genes, which he designated dw_1 and dw_2 .

No information is available on the possible relationship of the dw gene to either the dw_1 or the dw_2 gene.

Folded Leaf

Schrock (48) and Stanford and Cleveland (52) described similar folded leaf mutants. Leaflets showing the trait are folded toward the adaxial

³ SORENSEN, E.L. December 11, 1962. [Personal correspondence.]



FIGURE 2.--Crinkled leaf trait.

side of the leaflet along the midrib (fig. 4). The two halves of the leaflet may adhere along their entire length or only at the apex. Expression of the trait, according to Stanford and Cleveland, is variable, with some plants having nearly 100 percent folded leaflets and others having only 25 percent. The folded leaf trait is expressed in the unifoliolate leaf, but trifoliolate leaves are more accurately classified. Stanford and Cleveland showed that the folded leaf trait was conditioned by a single recessive gene inherited in a tetrasomic manner and proposed the symbol *fo*. A deficiency of recessive types was noted in simplex and duplex families. Schrock reported that his folded leaf mutant was conditioned by a dominant gene. However, he studied only one segregating generation so it was not possible to differentiate disomic from tetrasomic inheritance.

Mottled Leaf

Stanford and Cleveland (52) described a leaf mottling that was characterized by a chlorotic condition of the leaf surface, interspersed with flecks of green (fig. 5). Genetic studies showed that mottled leaf was determined by a single recessive gene that was inherited tetrasomically. The gene symbol *mo* was proposed. All mottled plants had some normal green sectors and some had one or more normal shoots. These green



FIGURE 3.--A, dw dwarf seedling; B, normal seedling.

sectors and green shoots were shown to be the result of a high mutation rate of the *mo* gene and not of a variable expression of the trait. Mutations appeared to occur from mottled to normal. Reverse mutations were not observed.

Multiple Cotyledons

In a population of 18,845 seedlings from alfalfa varieties Ranger and Vernal at St. Paul, Minn., D. K. Barnes and L. J. Elling (data unpublished) observed that 0.64 percent of the plants had three cotyledons and approximately 0.06 percent had four cotyledons (fig. 6). Inbreeding of plants with multiple cotyledons for three to five generations increased the frequency of multiple cotyledons from 1.6 percent to as high as 88.9 percent in several lines. In addition, a few plants with five and six cotyledons were observed. Selfing plants with five and six cotyledons invari



FIGURE 4.--Folded leaf trait. (Courtesy Agronomy Department, University of California.)



FIGURE 5.--Mottled leaf trait. (Courtesy Agronomy Department, University of California.)

ably produced progenies with higher frequencies of multiple cotyledons than were obtained from selfing plants with four cotyledons. Likewise, plants with three and four cotyledons produced a higher frequency of plants with multiple cotyledons than did plants with two and three cotyledons, respectively. However, no plants were found that were homozygous for multiple cotyledons. Discussions with others working on this trait indicate that plants homozygous for multiple cotyledons have not been isolated.

Barnes and R. W. Cleveland (data unpublished) at University Park, Pa., found that cotyledon chlorosis appeared to be associated with multiple cotyledons in diploid alfalfa. Barnes and Elling (data unpublished) at St. Paul, Minn., found that cotyledon chlorosis also appeared to be associated with multiple cotyledons in several lines of tetraploid alfalfa.

Pale-Green Plant Color

Barnes and Hovin (7) studied a mutant with pale-green stems and leaves (fig. 7). Chemical analyses of mutant plants indicated they had an increased ratio of chlorophyll a to chlorophyll b and were about 70-percent deficient in total chlorophyll. Seedling viability appeared normal when classified at 2 or 3 weeks after emergence. Mature pale-green mutants produced flowers and seed but often flowered several weeks later than normal siblings. Pale-green plant color was inherited tetrasomically and conditioned by the nulliplex condition of the pg gene. This mutant can be phenotypically differentiated from the viridis leaf color mutant described by Childers and McLennan (17) because the leaves and stems are uniform in color and have no white leaf blades or dark-green leaf veins.



FIGURE 6.--A, Chlorotic seedling having three cotyledons; *B*, normal green seedling having four cotyledons



FIGURE 7.--A, Plant having pale-green plant color; B, plant having normal color.

Ruptured Epidermis

Whittington and Burrage (56) described the ruptured epidermis trait as V-shaped markings on the lamina (fig. 8). The V-marked areas were light green, which resulted from the rupture of the leaf epidermis during expansion. The rate of respiration of the entire leaf was not duly affected by the markings and mutant plants were not noticeably weaker. It was proposed that the mutant trait was expressed when two or more recessive alleles of the tetrasomically inherited ru gene were present. The authors observed, however, that the mutant gene appeared to vary in penetrance and expressivity.

Seedling Lethal

Lewis and Elling (32) reported lethal genes associated with the death of seedlings 4 to 12 weeks old at the three- to four-leaf stage. When the seedlings were several weeks old, the new leaves were smaller than normal, and lighter in color, and had a folded appearance (fig. 9). Defoliation occurred gradually, beginning with the lower leaves. Stems were spindly and roots were underdeveloped. The trait appeared to be controlled by two complementary genes *Le* and *Le*₂, but the data were not sufficient to differentiate disomic from tetrasomic inheritance. However, later studies by Stringham and Elling⁴ indicated that the two genes were inherited in a tetrasomic manner. Plants of the *Le* --- *le*₂ *le*₂

⁴ STRINGHAM, G. A., and ELLING, L.J. March 19, 1965. [Personal communication.]



FIGURE 8.--Leaf showing characteristic banding of ruptured epidermis trait. (Courtesy School of Agriculture, University of Nottingham, Loughborough, England.)

Stem Color

Many workers have observed alfalfa plants with exceedingly red stems. Red stems are especially conspicuous in the alfalfa variety Moapa. According to Schaeffler (47) and to Korohoda (31) as cited by Atwood and



FIGURE 9.--A and *C*, Seedlings showing spindly stem and underdeveloped root characteristics of seedling lethal trait; B, normal seedling of same age. (Courtesy Department of Agronomy and Plant Genetics, University of Minnesota.)

Grun (2), crosses between red- and green-stemmed plants indicated that green was dominant over red. However, expression of red was often variable. Neither worker presented conclusive evidence as to genetic control. Expression of red stem color appears to be similar to that of most plant anthocyanins in that the degree of pigmentation is influenced by environment.

Sticky Leaf

Stanford (50) described a sticky leaf abnormality that was characterized by adhesion of adaxial sides of adjacent leaflets, as well as adhesion of opposite halves of the same leaflet (fig. 10). The trait can be identified in the three-leaf seedling stage. Sticky leaf plants are fertile. Stanford (51) indicated that the expression of sticky leaf was controlled by one gene with tetrasomic inheritance and was expressed only in the nulliplex condition. The symbol *st* was proposed.

Two Unifoliolate Leaves

The normal developmental sequence of an alfalfa seedling includes a pair of cotyledons, a single unifoliolate leaf, and trifoliolate leaves. Perga-ment (44) described alfalfa seedlings with two individual unifoliolate leaves instead of one. Preliminary data indicated that this trait (two uni-foliolate leaves) was conditioned by one recessive gene inherited in a tetrasomic manner. Later work by Davis,⁵ however, indicated that segregation ratios may fit either a tetrasomic-disomic or a strictly tetra-somic type of inheritance.

Viridis Leaf Color

Childers and McLennan (17) stated that this chlorophyll mutant lacked plastids in the leaf blade, although some plastids occurred in the mesophyll tissue around the midrib. Low and intermediate chlorophyll-producing classes of segregates could not be distinguished in the seedling stage, but classes could be differentiated when plants matured (fig. 11). In the low chlorophyll-producing class, petioles and veins were green and leaf blades were white. In the intermediate class, petioles and veins were green, but leaf blades were pale yellow green. The authors concluded that inheritance could be described on the basis of a single recessive gene inherited tetrasomically. They proposed the symbol v_l , This trait is phenotypi-cally different from the mottled leaf trait reported by Stanford and Cleveland (52).

Yellow Leaf

According to Childers (15), this mutant has yellow cotyledons (fig. 12) and leaves. A few seedlings lived to maturity but were dwarfs. Leaf

⁵ DAVIS, R.L. November 30, 1962. [Personal correspondence.]



FIGURE 10.---Sticky leaf trait. (Courtesy Agronomy Department, University of California.)



FIGURE 11.--*Top* and *center rows*, Leaves showing chlorotic leaf blades, green petioles, and green veins, characteristics of viridis leaf color trait; *bottom row*, normal leaves. (Courtesy Central Experimental Farm, Canada Department of Agriculture, Ottawa, Ontario.)



FIGURE 12.--Seedlings segregating for normal green cotyledons and yellow cotyledons. The latter are typical of the phenotype expressed by any of the x_1 , x_2 , x_3 , x_4 , and x_5 genes. (Courtesy Central Experimental Farm, Canada Department of Agriculture, Ottawa, Ontario.)

tissue structure of mutant plants was similar to that of normal green leaves, but plastids took up stain weakly in comparison with plastids of normal leaves. The trait was conditioned by a single recessive gene with tetrasomic inheritance. The gene was designated xantha-1 and assigned the symbol x_1 . Childers pointed out the similarity of this mutant to the

lethal yellow leaf seedling trait reported by Kirk (*30*), which the latter interpreted as controlled by two or more recessive genes with disomic inheritance. Childers recalculated Kirk's data and found that they supported his hypothesis of one recessive gene inherited tetrasomically.

Yellow Cotyledons

Dessureaux (22) reported four genes associated with yellow cotyledons in alfalfa. Seedlings with yellow cotyledons usually died before the unifoliolate leaf appeared. All four genes were associated with similar phenotypes and were inherited in a tetrasomic manner. The nulliplex condition of each gene produced yellow cotyledons. Phenotypes of the genes were similar to the xantha-1 gene described by Childers (15). Therefore, Dessureaux designated the four genes xantha-2, -5, -4, and -5 with gene symbols x_2 , x_3 , x_4 , and x_5 , respectively. Available evidence suggested a linkage between the x_2 and x_3 genes.

Zebra Leaf

According to Stanford (50), this abnormal leaf trait was characterized by a chlorotic stripping of the leaf, running at right angles to the midrib (fig. 13). It was readily identified under all environmental conditions, but



FIGURE 13.--Zebra leaf trait. (Courtesy Agronomy Department, University of California.)

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was more obvious at low temperatures when the chlorotic areas were more sharply defined. The demarcations were less pronounced at summer temperatures but could be identified. Zebra plants were noticeably weaker and differed in viability. A single tetrasomically inherited gene, designated *ze* by Stanford, controlled the zebra leaf trait. The nulliplex and simplex genotypes expressed the mutant phenotype. Dominance of the normal phenotype was expressed at the duplex level. The gene behaved primarily as a recessive except for the mutant expression of the simplex genotype.

Flower and Seed Traits

Branched Raceme

The branched raceme trait was first noted by Westgate (55). Later Dudley and Wilsie (23, 24) described a mutant plant that bore profusely branched panicle-like inflorescences instead of normal racemes, but had



FIGURE 14.--*A*, Branched raceme having vestigial flowers; *B*, racemes having normal flowers. (Courtesy Agronomy Department, Iowa State University.)

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primarily all vestigial floral parts (fig. 14). They proposed symbols *A*, *Bt*, *Ra*, and *Ra* for genes controlling inheritance of branched raceme. Genes *A* and *Br* were assumed to be inherited in a tetrasomic manner with random chromosome segregation; *Ra* and *Ra* were postulated as duplicate disomic genes. Some interactions among genes were observed. Branched raceme and vestigial flower were related genetically, but the basis for this relationship could not be determined.

Childers⁶ discovered a plant with branched racemes and normal floral parts among S_1 progenies of a plant tracing to the alfalfa variety Flamande. Normally the pedicels of a raceme produce one floret, but the pedicels of the branched raceme trait grew much longer than normal and each produced a cluster of from three to seven florets (fig. 15). As many as 119 florets were observed on a single branched raceme at Ottawa, Canada. Plant growth type was normal until the atypical flower traits developed. In the greenhouse, some florets aborted, but seed production generally was normal. When the branched raceme mutant was crossed with a normal plant, all F_1 plants had normal racemes. F_2 segregation indicated genetic control by a single recessive gene (br_1) inherited in a tetrasomic manner.

Exposed Stigma

According to Markus and Wilsie (35), this floral abnormality is characterized by complete exposure of the stigma, which is first observable in the late bud stage (fig. 16). Petal size is reduced and the keel never completely encloses the sexual column. The style appears normal in length, the filaments appear short, and the anthers shrivelled and non-dehiscent. Pollen grains are few and sticky, but appear normal in acetocarmine smears. In the greenhouse the clone was male sterile but completely female fertile. Under field conditions it did not set seed, apparently because it was unattractive to pollinating insects. The authors postulated a single recessive gene *es* inherited in a tetrasomic



FIGURE 15.--A, Normal raceme; *B*, branched raceme in bud stage; *C*, branched raceme in full bloom. (Courtesy Central Experimental Farm, Canada Department of Agriculture, Ottawa, Ontario.)

⁶ CHILDERS, W.R. February 13, 1964. [Personal correspondence.]



FIGURE 16.--A, Raceme with normal florets; *B*, raceme with florets having exposed stigmas characteristic of *es* gene. (Courtesy Agronomy Department, Iowa State University.)

manner. The designation a was used in the original publication but was not intended as a genetic symbol. The symbol *es* was suggested by Wilsie.⁷ The exposed stigma was expressed by the nulliplex condition. Consistent deficiencies of the nulliplex genotype suggested partial selection against recessive gametes.

A similar kind of exposed stigma was described by Nielsen (*36*). It was characterized by the stigma and anthers growing through the tip of the keel petal (fig. 17). The stress on the staminal column was less, so that characteristic bending to about 90 degrees, which takes place after tripping in normal flowers, failed to occur. Thus, the staminal column of the abnormal flowers was straight, and after tripping turned slowly toward the standard. Expression of the exposed stigma trait was greatest when the air was relatively warm and dry, and least when the air was cold and humid. Flowers with exposed stigma could be pollinated by bees without tripping. When tripping occurred, the bees did not receive the hard blow from the staminal column usually experienced from tripping normal flowers. Preliminary studies indicated that the exposed stigma trait was heritable but complex, because expression of the trait was variable and greatly affected by environmental conditions.

Flower Color

More than 20 studies of flower color inheritance in tetraploid alfalfa have been published. Information from them and from studies that have not been published was summarized and reevaluated by Barnes (3). A summary follows but for sake of brevity, the original references are not cited.

⁷ WILSIE, C.P. November 27, 1962. [Personal correspondence.]

Purple.--Purple flower color pigments were shown to be controlled by one gene (*P*) with tetrasomic inheritance. Presence of one or more dominant alleles produced a purple-flowered phenotype.

*Yellow.--*Only limited data were available concerning the inheritance of yellow flower color pigments in tetraploid alfalfa. The available data could be interpreted either as disomic or tetrasomic inheritance. However, a pattern of tetrasomic inheritance for several genes (Y_1 and Y_2) with accumulative effects was favored.



FIGURE 17.--Raceme having florets with exposed stigmas. Genetic control of this trait has not been investigated. (Courtesy Department of Plant Culture, Royal Veterinary and Agricultural College, Copenhagen, Denmark.)

Variegated.--Usually all F_1 and many F_2 and F_3 progenies from crosses between purple- and yellow-flowered plants have variegated flowers. This has been shown to be due to a copigmentation of the purple pigments in the epidermal layer of the flower with a background of yellow pigments. Gene dosages for purple, yellow, and modifying pigments, and age of flowers were shown to be factors that influenced degree of variegation.

White.--White-flowered plants were described as being devoid of anthocyanin pigmentation in flowers, seeds, stems, leaves, and roots. The white-flowered phenotype was produced by the homozygous recessive condition of the color-conditioning c gene. The *cccc* genotype is epistatic over the P gene controlling purple flower color and the *Rd* gene controlling red root (3).

*Cream.--*Cream flower color was due to the homozygous recessive condition of the several genes controlling yellow flower color and the *P* gene controlling purple flower color. The genotype of cream-flowered plants did not alter color of any other plant organ. Cream-flowered plants were readily differentiated from white-flowered plants by the presence of pigmented floral veins, anthocyanins in the stems, and tan or yellow seeds.

Except for effects of gene dosage, diploids and tetraploids are very similar with regard to the inheritance of purple, yellow, white, and cream flower color. A better understanding of yellow flower color inheritance and the effects of modifying factors can be obtained from diploids. (See p. 33.)

Hornless Wing Petals

This abnormality is characterized by wing petals that fold around the keel instead of extending normally (fig. 18) and is caused by the absence of horns on wing petals. Preliminary genetic analyses by Barnes, A. W. Hovin, and. Cleveland (data unpublished) at University Park, Pa., indicated that the length of the wing petal horns appeared to be controlled by one tetrasomically inherited gene hl with partial dominance. The wing petals of the nulliplex genotype were hornless and enclosed the keel petals. The phenotype of the simplex was similar to the nulliplex except for the presence of short (0.75 to 1.25 millimeters) horns. The wing petals of duplex plants were approximately one-half extended and had horns 1.25 to 1.65 millimeters long. Triplex and quadruplex genotypes were difficult to differentiate because both had normally extended wing petals and horns of similar lengths (about 1.75 millimeters). A consistent deficiency of nulliplex plants was observed. The apparent ability to identify most genotypes on the basis of phenotypes may make this a useful gene marker for many studies. However, its usefulness in pollination studies will be limited because most pollinating insects are unable to trip flowers of nulliplex or simplex plants.

Male Sterility (Anther Nondehiscence)

A male sterile (nondehiscent) alfalfa plant was described by Childers (14). The sterility was characterized by a precocious development and overgrowth by the tapetal tissue, which was associated with an extreme vacuolation of the cytoplasm of the adjacent sporogenous cells. This



FIGURE 18.--Hornless wing petal trait: *A*, Portion of wing petal showing complete lack of wing hooks; *B*, portion of wing petal showing normal length wing hook; *C*, phenotype of floret with nulliplex genotype for *hl* gene conditioning hornless wing petals; *D*, normal floret quadruplex for *hl* gene.

leads to a degeneration of the pollen mother cells in the early prophase stage. Heavy-walled pollen grains were sometimes present at maturation of anthers, but the shrunken nature of the anthers precluded the possibility of anthesis. Inheritance of this nondehiscence type of sterility was attributed to action of duplicate genes (ms_1 and ms_2) with disomic inheri-

tance. Plants having a homozygous recessive genotype, $ms_1 ms_1 ms_2 ms_2$, were nondehiscent, but the presence of one or more dominant alleles at either the Ms_1 or Ms_2 locus restored ability of anthers to dehisce. Childers indicated that because F₃ populations were not included in the study, no attempt was made to determine whether tetrasomic inheritance was operative.

Male Sterility (Pollen-Grain Degeneration)

Childers and McLennan (16) described an alfalfa plant that was completely male sterile under greenhouse and field conditions. Male sterility was characterized by an atypical behavior after microspores were released from tetrads. Complete degeneration of microspores followed. The stage at which this degeneration occurred was later than the stage at which the nondehiscent type of male sterility reported by Childers (14) occurred. Function of the female gametophytes of plants with degenerate microspores was normal. Genetic studies indicated that complete male sterility was controlled by three recessive genes, which were probably inherited in a disomic manner. A tetrasomic interpretation was not discounted, however. No cytoplasmic factors were associated with this type of male sterility. Subsequently, McLennan and Childers (33) transferred this male sterility from the tetraploid to the diploid level and showed that the trait was controlled by one gene ms_3 instead of three disomic genes. (See p. 33.) Thus, they concluded that one gene with tetrasomic inheritance was responsible for this form of male sterility in tetraploid alfalfa.

Open Keel

Nielsen (36) described alfalfa flowers with abnormally open keels and longitudinal separation of keel petals (fig. 19). Expression of this condition is best at relatively low humidities and high temperatures. Most flowers with the open keel trait are more easily tripped by honey bees than are normal flowers. Preliminary studies indicated that inheritance of the open keel trait is probably simple.

Pod Hairiness

According to Armstrong and Gibson (1), pods of *Medicago glutinosa* Bieb. are characterized by upright hairs, each hair exuding a sticky globule from the tip. Armstrong and Gibson crossed *M. glutinosa* with a plant of *M. media* Pers. that had appressed pod hairs. The *M. glutinosa* traits were dominant in the F_1 generation. F_2 families segregated in ratios of either 3 upright: 1 appressed, or approximately 3 upright: 4 intermediate: 9 appressed. Armstrong and Gibson postulated that M. *glutinosa* carried a dominant factor *G* for upright hair posture, and that the *M. media* parent was homozygous for the recessive g allele and heterozygous for a factor *I*. It was also postulated that the *I* allele partly inhibited the expression of *G*. Thus, the proposed genetic constitution of the *M. glutinosa* and *M. media* parents were *GGii* and *ggli*, respectively. Inheritance was based on a disomic scheme. The authors did not test for tetrasomic inheritance.

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FIGURE 19.--Raceme with flowers showing open keel trait (arrow). (Courtesy Department of Plant Culture, Royal Veterinary and Agricultural College, Copenhagen, Denmark.)

Seed Color

MacVicar (34) noted that seed color of alfalfa was predominantly a bright greenish yellow but that density of color ranged from a very light yellow to orange or light brown. Exceptional plants with true black or true white seeds were isolated. A black-seeded plant was discovered in the third generation selfed line of variety Grimm.

*Black.--*F₂ studies by MacVicar indicated that white-seeded plants were homozygous for a recessive factor *y*, which resulted in the absence of yellow pigment. Inheritance of the black-seeded trait was fairly complex, requiring a hypothesis of at least three factor pairs. Black color was attributed to a single gene mutation, whose expression was affected by two modifying genes~ The genotype of the original black-seeded parent was postulated to be $YYC_1c_1C_2c_2Bb$, where the genes are represented by *Y* for yellow pigment, *B* for black pigment, and C_1 and C_2 for factors that modify the expression of black. Deficiencies, which were attributed to elimination by gametic or zygotic lethals, were noted in black and deep mulatto classes. No reference was made as to the possibility of tetrasomic inheritance. Seed coat color was affected somewhat by environment.

White.--Alfalfa plants with white .seeds were described by MacVicar (*34*), Oldemeyer (*38*), Risius (*46*), Stanford (*49*), and Waldron (*54*). The mutant plants had albino-white or creamy-white seeds, which were distinctly different from normal tan or yellow seeds (fig. 20). Plants with white seeds had white flowers and lacked anthocyanin pigments in foliage.

Waldron stated that white seed color was inherited in the same manner as white flower color. MacVicar reported that white seed color was recessive to tan and that the difference was conditioned by either one or two genes with disomic inheritance. Oldemeyer studied seed color inheritance in F_2 and backcross generations of crosses between plants having white seeds and white flowers and both plants having tan seeds and purple flowers and plants having tan seeds and yellow flowers. He concluded that white seed color was produced only when a color gene c was in the homozygous recessive condition and when a dominant allele of an inhibitor gene was present. However, neither MacVicar nor Oldemeyer grew the critical generations needed to positively establish the number of genes and type of inheritance. Risius and Stanford later demonstrated conclusively that white seed color and white flower color were both controlled by the same single recessive gene (c) with tetrasomic inheritance.



FIGURE 20.--A, Normal yellow seeds; B, white seeds.



FIGURE 21 .--Variations in floral structure produced by vestigial corolla trait. (Courtesy Central Experimental Farm, Canada Department of Agriculture, Ottawa, Ontario.)

Vestigial Corolla

A floral mutant with a variable corolla (fig. 21) was isolated by Pankiw and Bolton (39) in the S_1 progeny from an alfalfa variety Vernal plant with high seed set. Nectar production was below that of the parent. The nectary was at the base of the staminal column in some florets, but was below the anthers in others. Pollen production of this mutant was limited but pollen was viable. Self-fertility was low but cross-fertility normal. Honey bees visited the mutant flowers and some seed set resulted. Pankiw and Bolton (40) studied the seed-production possibilities of this mutant and concluded that the various types of vestigial florets produced might be useful for improving pollination by honey bees.

Pankiw and Goplen (41) reported that the vestigial corolla trait was controlled by a single recessive gene (vc) with tetrasomic inheritance. The expression of the vc gene was subjected to considerable environmental influence.

Vestigial Flower

Dudley and Wilsie (23) described a mutant plant with profusely branched, panicle-like inflorescences (branched raceme) and vestigial floral parts. Only an occasional petal was normal sized (fig. 14). Seed setting was not observed to occur in the field. Genetic studies indicated that the branched raceme and vestigial flower traits were related, but the basis for the relationship could not be determined by the data.

In a subsequent publication (24) the same authors proposed symbols D, V, F, and F' for genes associated with the vestigial flower trait. D and V were assumed to be tetrasomic genes with random chromosome segregation; F and F' were assumed to be duplicate disomic genes. Some genic interactions were noted. The authors concluded that the vestigial flower trait would be of little value as a genetic marker because of the difficulty in making crosses and the complexity of genetic mechanisms involved.

Plant Resistance

Downy Mildew Resistance

Infected leaves of alfalfa plants attacked by the downy mildew fungus *Peronospora trifoliorum* DBy. become yellow. Downy mildew causes significant leaf drop and:seedling damage during cold, wet weather. Jones and Smith (28) suggested that susceptibility was dominant to resistance. Pedersen arid Barnes (43) reported that resistance to downy mildew appeared to be due to one tetrasomically inherited gene with incomplete dominance. This gene was designated *Dm*. The level of resistance appeared to be determined by accumulative gene action with genotypes as follows: Very susceptible plants = nulliplex genotype; moderately susceptible=simplex; moderately resistant = duplex; and resistant = triplex or quadruplex. It was suggested that conflicting reports as to the mode of inheritance of downy mildew resistance in alfalfa might be explained by the existence of biological races of *P. trifoliorum*.

Pea Aphid Resistance

The pea aphid *Acyrthosiphon pisum* (Harris) causes yellowing and stunting of alfalfa. Jones, Briggs, and Blanchard (29) determined the inheritance of resistance in plants tracing to the variety Chilean. They caged second-instar nymphs and observed rate of nymphal production and length of production period. On the resistant parent, second-instar nymphs usually failed to reach maturity in the normal time and upon maturity produced few, if any, nymphs. The length of life did not exceed 9 days. On the susceptible parent, from 4 to 9 nymphs were produced per day for a period of 10 to 15 days. F₂ and F₃ segregations indicated that resistance was controlled by one dominant gene *Pa* and one recessive gene *pr*. The designations A and b were used in the original publication but were not intended as genetic symbols. The symbols *Pa* and *pr* were suggested by F. N. Briggs.⁸ Both genes were assumed to be inherited in a disomic manner. The proportion of homozygous to heterozygous families suggested a linkage between the two genes with a crossover value of about 28 percent. The authors were unable to explain segregation on the basis of random chromosome segregations of an autotetraploid.

Recent reports have shown that plant reaction to the pea aphid may be affected by environmental conditions.

Root-Knot Nematode Resistance

Goplen and Stanford (25) used nematode reproduction (presence of egg masses) as criterion for classification of resistance to root knot (fig. 22), a disease caused by species of the genus *Meloidogyne* Goeldi, 1887. Root galling and proliferation are common symptoms of the disease. Studies of resistance in two selections from the variety Vernal showed that resistance to *M. hapla* Chitwood, 1949, was controlled by one domi-

⁸ BRIGGS, F.N. March 23, 1964. [Personal correspondence.]



FIGURE 22.--A and C, Plants susceptible to root-knot nematode; B, plant resistant to root-knot nematode.

nant gene Rk_1 with tetrasomic inheritance. Limited genetic data from two other plant selections suggested that one or two additional genes also conferred resistance. Similar inheritance studies demonstrated that resistance to *M. javanica javanica* (Treub, 1885) Chitwood, 1949, was controlled by one dominant, tetrasomic gene Rk_2 and possibly a second gene. The Rk_1 and Rk_2 genes were linked, but an estimate of linkage intensity was not obtained.

Stem Nematode Resistance

Alfalfa seedlings attacked by the stem nematode *Ditylenchus dipsaci* (Kuhn, 1857) Filipev, 1936, develop a swollen cotylendonary node and swollen and stunted leaf petioles (fig. 23). Older plants have swollen stems, shoots, and petioles, as well as distorted leaves and petioles. Grundbacker and Stanford (26) reported that resistance in an Iranian introduction, P.I. 141462, and possibly in the variety Talent was conditioned by one dominant gene with tetrasomic inheritance. The resistant gene in the Iranian selection was designated *Sn* by Stanford.⁹ Grundbacker and Stanford (26) reported a second type of resistance controlled by one or more minor genes in introductions from Argentina and in resistant selections from the variety DuPuits. Segregation in progenies from crosses of the variety Lahontan could not be interpreted on a factorial basis. Controlled temperatures were necessary because expression of nematode symptoms was temperature sensitive. Numbers of nematodes and eggs were used as criteria of resistance. Previous studies on stem nematode resistance by Burkart (12) and Ragonese and Marco (45) suggested a multifactorial system.

⁹ STANFORD, E.H. July 10, 1963. [Personal correspondence.]



FIGURE 23.--A and B, Seedlings susceptible to stem nematode; C, seedling resistant to stem nematode.

DIPLOID ALFALFA (2N=16) Leaf and Stem Traits

Cotyledon Chlorosis

Barnes and Cleveland (data unpublished) at University Park, Pa., observed that seedlings from a cross between two unrelated hybrids of *Medicago sativa* and M. *falcata* segregated for green, yellow, and white cotyledon color (fig. 24). Genetic studies indicated that color was controlled by two independent genes gc and Yc, which segregated in an F₂ ratio of 12 green: 3 yellow : 1 albino. The dominant allele of the gc gene was epistatic to both alleles of the Yc gene and produced green cotyledons. In the absence of a dominant gc allele, the dominant Yc allele produced yellow cotyledons. Seedlings that were homozygous recessive for both genes were albino and died a few days after emergence. Yellow seedlings died within several weeks. In addition, either gametic or zygotic, or both, inviabilities seemed to have caused about a 33percent deficiency of yellow seedlings and about a 50-percent deficiency of albino seedlings.

An association was noted between chlorotic seedlings and multiple cotyledons. In the original cross, multiple cotyledons were observed on about 70 percent of the albino seedlings, 10 percent of the yellow seedlings, and only 1 percent of the green seedlings.



FIGURE 24.--A, Normal green seedling; B, yellow seedling; C, albino seedling.

Crinkled Leaf

A crinkled leaf trait similar to that shown in figure 2 was observed by Barnes and Cleveland (data unpublished) at University Park, Pa., in the advanced generations of a cross between diploid *M. sativa* and diploid *M. falcata*. This trait appeared to be phenotypically similar to the one reported in tetraploid alfalfa by Odland and Lepper (*37*). In clones having a severe expression of the trait, the crinkling was apparent at all stages of growth, but crinkling in clones with a less severe expression became most apparent on the early aftermath growth of established plants. F₂ progenies segregated 13 normal to 3 crinkled, indicating that crinkled was controlled by two genes e_1 and Cr_1 . (The e_1 and Cr_1 gene symbols were selected in order to correspond with *E* and *Cr* symbols suggested by Odland and Lepper.) Presence of at least one dominant allele at each of the two loci or the homozygous recessive condition of the alleles at the Cr_1 locus were dominant and the e_1 locus was homozygous recessive.

Lutescent Cotyledons

Barnes and Cleveland (data unpublished) at University Park, Pa., found seedlings in advanced generations of diploid *M. sativa* \times diploid *M. falcata* hybrids with yellow-green cotyledons at emergence. Several days later the color of the cotyledons usually darkened in the vicinity of the cotyledonary axis and veins (fig. 25). About I week after emergence,



FIGURE 25.--A, Newly emerged lutescent seedling; B, cotyledons becoming darker green; C, normal green cotyledons. Chimeral sectoring usually is present in unifoliolate leaves.

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the cotyledons became normal green throughout. Lutescent cotyledon appeared to be controlled by two complementary genes Lc_1 and Lc_2 . Plants with the genotypes Lc_1-Lc_2 -had normal green cotyledons; plants with $Lc_1-lc_1 lc_2$, $lc_1 lc_1 Lc_2$ --, or $lc_1 lc_2 lc_2 lc_2$ genotypes had yellow-green cotyledons. All genetic data were obtained from cotyledon color. However, unifoliolate and trifoliolate leaves usually had a sectoring trait (fig. 25). The degree of sectoring varied; some plants had slight sectoring on only the lower leaves; others had sectoring throughout. It was assumed that sectoring was attributable to the same genetic control as the yellow cotyledon color.

Multifoliolate Leaves

Bauder (8) reported that some tetraploid alfalfa plants had multi-foliolate leaves instead of the normal trifoliolate leaves. Bingham (9) found plants with this trait in both diploid and tetraploid populations (fig. 26). Leaflet numbers ranged from three to nine among tetraploids and three to six among diploids. Diploid genetic studies indicated that a recessive gene (mf) in the homozygous condition was necessary for the production of multifoliolate leaves. Penetrance ranged from incomplete for plants with predominately trifoliolate leaves to complete for those with predominately multifoliolate leaves. Differences in penetrance were explained on the basis of two additive genes with three alleles for each gene. Bingham and Murphy (10) reported that this trait was highly heritable in tetraploid alfalfa and the mode of inheritance appeared similar to that at the diploid level.



FIGURE 26.--Multifoliolate trait in diploid alfalfa. Expression is similar in tetraploid alfalfa. (Courtesy Department of Plant Breeding, Cornell University.)

Flower and Seed Traits

Calyx Pigmentation

Clement¹⁰ observed flecks of anthocyanin pigmentation (fig. 27) on the calyx of some diploid *M. falcata*, diploid *M. sativa*, and tetraploid M. *sativa* plants. The trait corresponds closely to one described by Schrock (48) in tetraploid plants. Inheritance studies in diploid alfalfa by Clement demonstrated that calyx pigmentation was governed by a single dominant gene Cp. Preliminary data in tetraploid alfalfa indicated that pigmentation is probably determined by a single dominant gene inherited in a tetrasomic manner; however, data on critical generations are needed before this hypothesis can be established.



FIGURE 27.--Flower buds showing characteristic flecking of calyx pigmentation phenotype.

¹⁰ CLEMENT, W. M., JR. December 14, 1962. [Personal correspondence.]

Flower Color

Results of studies of the inheritance of flower color in diploid alfalfa have been published by Buker and Davis (11), Cooper (18), Cooper and Elliott (19, 20), and Twamley (53). The information from these studies, as well as unpublished data from other research workers, was summarized by Barnes (3). A digest of genetic mechanisms conditioning flower color follows. For the sake of brevity, further reference is not made to original papers.

Purple.--Chemical analyses of diploid alfalfa indicated that purple flower color was due to three anthocyanin pigments inherited as a unit and controlled by one dominant gene (*P*).

*Yellow.--*Yellow flower color pigments in diploid alfalfa were identified as being primarily xanthophyll with a small amount of β carotene present. Genetic data suggested that yellow flower color was controlled by at least three and probably four genes with accumulative effects. Two of the genes controlling xanthophyll were designated Y_{x_1} and Y_{x_2} . The other two genes were designated Y_3 and Y_4 but were not identified with any specific pigment.

*Variegated.--*All F_1 and many F_2 and F_3 progenies from crosses between purple- and yellowflowered plants have variegated flowers. This has been shown to be due to a copigmentation of the purple pigments in the epidermal layer of the flower with a background of yellow pigments. Degree of variegation was shown to be influenced by dosages of purple, yellow, and modifying pigments, and by age of flower.

*Cream.--*The homozygous recessive condition of the four genes conditioning yellow flower color and the *P* gene produces a cream flower color. The genotype of the cream-flowered plants did not appear to alter the pigmentation of other organs of the plant.

*White.--*White-flowered plants were completely devoid of anthocyanin pigmentation in flowers, seeds, stems, leaves, and roots. The white phenotype was produced by the homozygous recessive condition of the color-conditioning c gene, which is known to be epistatic to the P and Rd genes.

*Modifying pigments.--*Nine anthoxanthin pigments were chemically identified in diploid alfalfa. Three of the anthoxanthins were kaempferol glycosides; six were quercetin glycosides. None of the pigments appeared to impart a phenotypically significant color of its own, but each tended to have a modifying effect when copigmented with anthoxyanin or xantho-phyll. The inheritance of production of two kaempferol glycosides was controlled by two dominant independent genes designated K_2 and K_3 .

No association or linkage was detected among the *P*, Yx_1 , Yx_2 , K_2 , and K_3 genes in diploid alfalfa.

Male Sterility (Pollen-Grain Degeneration)

McLennan and Childers (33) transferred the completely male sterile trait, previously described on page 22, from the tetraploid to the diploid level by crossing the male sterile tetraploid with diploid forms of *M. sativa*, *M. falcata*, and *M. hemicycla* Grossh. to produce triploids. Backcrosses to the diploid species produced 16 chromosome plants, two-thirds of which produced selfed progenies segregating 3 : 1 for male sterility. Further F_2 and backcross data showed that the complete male sterility factor was governed by a single recessive gene ms_3 . These data indicated that this form of male sterility in tetraploids was conditioned by a single gene inherited in a tetrasomic manner instead of three disomic genes as originally postulated.

Ovule Number

Significant differences for average ovule number per floret were observed by Barnes and Cleveland (5) in plants of tetraploid and diploid alfalfa (fig. 28). The average number of ovules per floret was relatively constant among racemes of the same plant, but differed slightly among florets of the same raceme. In diploid alfalfa, the average ovule number per floret segregated into 5 distinct ovule number classes, which averaged approximately 7.0, 8.5, 10.0, 11.5, and 13.0 ovules. Four genes with accumulative



FIGURE 28.--A, Ovary having 6 ovules; B, ovary having 17 ovules.

effects were assumed for ovule number. Three of these genes, Ov_1 , Ov_2 , and Ov_3 showed nearly complete dominance, while the fourth, Ov_4 , expressed incomplete dominance. The 7.0-ovule class was attributed to the homozygous recessive condition of all four genes. The presence of one or more dominant alleles at any one of the four loci raised the average ovule number per floret approximately 1.5 ovules.

Purple Bud Color

Many yellow-flowered alfalfa plants exhibit anthocyanin pigment at the bud tip (fig. 29). This pigment usually fades at anthesis. The occurrence of purple bud color in yellow-flowered plants has been reported only in progenies of the cross, purple-flowered *M. sativa* X yellow-flowered M. *falcata*. Both Cooper (18) and Twamley (53) observed this bud-color pigment, but neither determined its mode of inheritance. Barnes and Cleveland (6) determined that purple bud color required the presence of



FIGURE 29.--A, Bud of yellow flower with purple anthocyanin pigments at tip; *B*, bud of yellow flower devoid of visible anthocyanin pigments. (Courtesy Agronomy Department, Pennsylvania State University.)

two dominant genes with complementary action and postulated genes *Bs* and *Bf*, one each from M. *sativa* and M. *falcata*, respectively.

Vein Color of Standard Petal

Darkly pigmented veins occur with varying intensity in the standard petal of most alfalfa flowers. The veins begin in the throat of the flower and extend toward the forward edge of the standard. Because either purple or green petal color reduces the prominence of floral veins, Barnes and Cleveland (6) determined the genetics of vein color in yellow-flowered diploid alfalfa. Some plants had veins throughout the standard petal; others were completely devoid of floral vein color (fig. 30). The occurrence of darkly pigmented veins in the standard was controlled by duplicate genes Vs_1 and Vs_2 . Presence of one or more dominant alleles produced darkly pigmented veins. The homozygous recessive condition of both genes was associated with absence of vein color. An association between pigmentation of veins in standard and wing petals was observed but was not interpreted genetically.



FIGURE 30.--A, Floret having pigmented veins: B, floret lacking pigmented veins. (Courtesy Agronomy Department, Pennsylvania State University.)

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