

3

The Genus *Medicago* and the Origin of the *Medicago sativa* Complex¹

CARLOS F. QUIROS

*University of California
Davis, California*

GARY R. BAUCHAN

*USDA-ARS
Beltsville, Maryland*

The most recent analysis of the genus *Medicago*, is the comprehensive taxogenetic study of Lesins and Lesins (40), which revised and expanded the chapter written by Lesins and Gillies (35) for *Alfalfa Science and Technology* (Agronomy monograph 15). In this chapter, we attempt to update and complement these two treatises focusing on recent research developments dealing with the origin and evolution of *Medicago*.

3-1 CENTERS OF DIVERSITY

The genus *Medicago* is very extensive comprising more than 60 different species, two-thirds of which are annuals and one-third perennials (40). *Medicago* is endemic to the Mediterranean region, spreading to Spain and to the Canary Islands toward the West, to People's Republic of China toward the East, to Siberia toward the North and to Yemen toward the South. The primary center for the genus is found in the Caucasus, northwestern Iran and northeastern Turkey (20).

3-2 PLOIDY

The basic genomic number of *Medicago* is $x=8$, except for the annual species *M. constricta* Dur., *M. praecox* DC., *M. polymorpha* L., *M. rigidula* (L.) All., and *M. murex* Willd. which have a genomic number of $x=7$. Cytological studies (41) indicate that the last four species might have arisen independently by chromosomal translocations involving two chromosomes and the subsequent loss of the resulting centric fragment.

¹ Dedicated to the memory of the late Professor Karl Lesins.

On the other hand, *M. constricta* seems to have evolved from *M. murex* by additional chromosomal rearrangements.

Three ploidy levels are found among the *Medicago* spp., diploids $2n=2x=14$ and $2n=2x=16$, tetraploids ($2n=4x=32$), and hexaploids $2n=6x=48$. Recently, Bauchan and Elgin (4) reported that the species *M. scutellata* (L.) Mill. and *M. rugosa* Desr. have $2n=30$. These might be allotetraploids resulting from the hybridization of $2n=14$ and $2n=16$ species, followed by polyploidization. Since the majority of the *Medicago* spp. are diploids, it is likely that the basic evolution of the genus has taken place at this level. The tetraploid species probably arose by unreduced gametes (46,51,78), giving rise to vigorous and highly heterozygous individuals, aggressive enough to colonize new habitats and surpass the range of distribution of diploids (55). Two alloautoploid hexaploids species have been reported, *M. cancellata* M.B. and *M. saxatilis* M.B., both perennials. Hexaploid accessions of the otherwise tetraploid species *M. arborea* L. have been reported (40). Presumably these are autohexaploids.

3-3 BREEDING SYSTEMS

Annual species of *Medicago* are autogamous, as a result of the presence of self-tripping in their flowers. In conformity with their breeding system, progenies of annual plants are remarkably uniform (57). Conversely, most perennial species are allogamous, with different degrees of self-incompatibility. Although some self-tripping occurs in these species, as a rule they rely on insect pollinators to activate their flower tripping and pollination (40). Most of these species are quite polymorphic, because of their high degree of outcrossing (54). Their pollinating agents include numerous species of bees.

3-4 EVOLUTION

According to Lesins and Lesins (40), the evolution of the genus took place during the Tertiary Period. Important geological events occurred at that time, including the formation of prominent mountain ranges, such as the Alps, Pyrenees, Apennines, Himalayas, and the Tien Shan. The northern coast of the Mediterranean appears to be the area of origin of perennial species based on their present range of distribution. During part of that time, the Mediterranean basin was a hot desert, resulting from the intermittent closure of Gibraltar. This provided new habitats for the evolution of annuals from the perennial species. The annual species with a short life cycle and seed dormancy may have colonized newly opened areas subject to intermittent flooding. Also, the change in longevity from perennial to annual, was accompanied by the self-pollinating trait, an essential reproductive strategy because of their isolation and lack

of pollinators in newly colonized habitats. The final opening of the Gibraltar resulted in the extinction of numerous species, leaving those that remained on mountain peaks, which now form several Mediterranean islands. The annual species, as a younger group, may not contribute much to understanding the area of origin of the genus. The shrub *M. arborea*, the oldest member of the genus, is genetically too distant in its present tetraploid and hexaploid forms to be considered an immediate progenitor of the diploid perennial species (40). *Medicago carstiensis* Wulf. may be a possible relic, because of its unique physiological and morphological traits (19). It thrives in shade and requires exposure to a cold treatment in order to flower.

3-5 TAXONOMY

3-5.1 Identification of the *Medicago* Species

3-5.1.1 Useful Traits to Distinguish Different Taxa

Legume and seed characteristics are considered of great taxonomic value in distinguishing *Medicago* spp. (40). Other important traits for taxonomic purposes are growth habit and longevity, organ hairness, inflorescences: bracts, stipules, and florets; leaves, cotyledons, and chromosome numbers. Lesins and Gillies (34) and Lesins and Lesins (40) provide excellent photographic records of most important morphological traits used in the identification of *Medicago* spp. On the basis of these traits, useful keys have been constructed as guides for species identification. However, these classification keys using macroscopic characteristics fail in several instances to group related taxa. Thus, *M. sativa* spp. *falcata* Arcangeli., although a subspecies of *M. sativa* L., is grouped with other less related perennials, because of its yellow flowers and uncoiled pods.

There seems to be enough distinction in the characteristics of pollen grains to use them as a criteria in differentiating many species (37) and related genera (68). Under the light microscope it is possible to distinguish two broad groups of pollen grains on the basis of shape: cylindrical, including spindle and block shapes, and pyramidal, including triangular and tetragonal bisphenoids. Electron microscopy reveals more subtle differences for each of these two categories. For example, *M. arborea* has cylindrical pollen grains (Fig. 3-1A), while *M. sativa* have elliptical grains (Fig. 3-1C). The grains of *M. rhodopea* Velen., although cylindrical, are wider at one end (Fig. 3-1E). Some accessions of *M. tornata* Mill. show wing-like protuberances in the colpi (Fig. 3-2A and 3-2B). Within the pyramidal shapes, the pollen of *M. soleirolii* Duby and *M. hybrida* Trautv. are good representatives of triangular pyramids (Fig. 3-1D and 3-1F), while that of *M. leiocarpa* Benth., is representative of tetragonal bisphenoids (Fig. 3-1B). Although differences in exine sculpting are evident

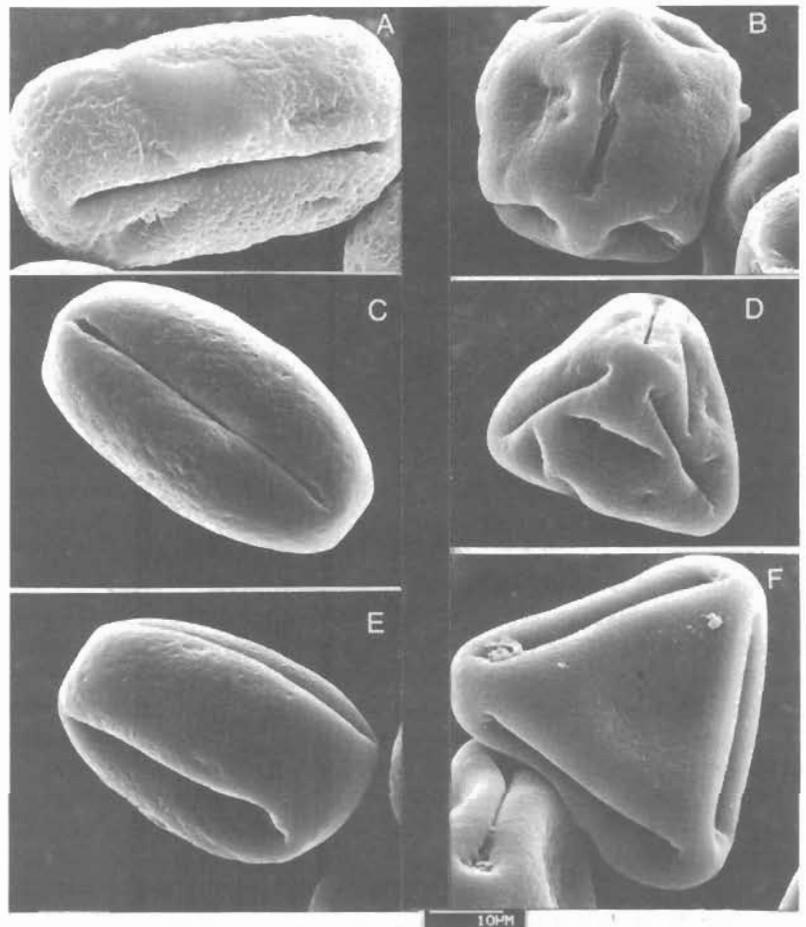


Fig. 1A–1F. Scanning electron microscope photographs of *Medicago* pollen grains, 1 cm = 10 µm. Fig. 1A. *M. arborea*, UAG 31, cylindrical. Fig. 1B. *M. leiocarpa*, UAG 555, tetrahedral bisphenoid. Fig. 1C. *M. sativa* ssp. *sativa* elliptical. Fig. 1D. *M. hybrida*, UAG 1637, pyramidal. Fig. 1E. *M. rhodopea*, UAG 493, cylindrical, with wider end. Fig. 1F. *M. soleirolii*, UAG 2399, pyramidal.

among various species and some accessions of the same species (Fig. 3–2C to 3–2F), they are not elaborate.

Numerical taxonomy has been applied to study the *Medicago* spp. Small (67) recognized 12 species groupings in the genus, by numerical taxonomic analysis based on 75 characters, mostly vegetative and pod traits. The classification of the annual *Medicago* spp. was in good agreement with taxonomic conclusions reached by other researchers, although several discrepancies were found. This might be due to the fact that Small conducted his study on herbarium specimens of plants grown in their natural habitats. Many traits are known to be influenced by environment, thus lacking constant expression, i.e., leaflet length and narrowness, height,

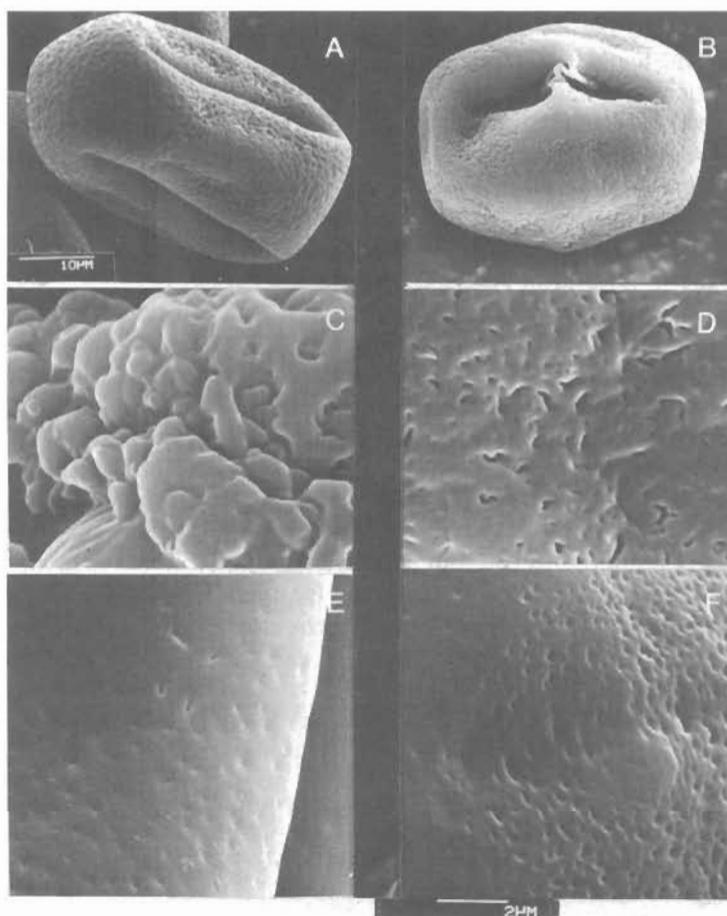


Fig. 2A–2F. Scanning electron microscope photographs of *Medicago* pollen grains. Fig. 2A–2B: 1 cm = 10 μ m, Fig. 2C–2F: 1 cm = 2 μ m. Fig. 2A. *M. tornata*, UAG 1590, cylindrical. Fig. 2B. *M. tornata*, UAG 1324. Fig. 2D. *M. sativa* ssp. *sativa* exine. Fig. 2E. *M. soleirolii* exine, UAG 2399. Fig. 2F. *M. leiocarpa* exine, UAG 555).

and other quantitative characteristics. Therefore, it is likely that part of the observed variation was environmental.

Small and Lefkovitch (73) attempted to discriminate the subspecies of *M. sativa* by agrochemotaxometry. That is, by numerical analysis involving morphological and chemical variables. They analyzed plants of each subspecies for various chemical elements and for amounts of structural components. They found significant differences in S and Ca content among subspecies, but this measurement was not as reliable in making separations on the basis of pod and flower characteristics. More recently, Small and Brookes (70) used canonical analysis to define intermediate types in the *M. sativa* complex. Numerical taxonomy has been used to reclassify the annual *Trigonella asclersoniana* Urb. as *M. hypogaea* Small under the section *Spirocarpus* (71). In another study, Small and Brookes

(72) reclassified the $x=8$ accessions of *M. murex* as *M. lesinsii* Small by discriminant analysis.

The ideal approach to elucidate taxonomic relationships in *Medicago* would involve the use of monogenic traits of constant expression in most environments. The technique of enzyme electrophoresis discloses many genetic markers that comply with these requirements. Furthermore, isozymes allow the detection of homozygous and heterozygous individuals, facilitating the determination of genetic variability and amount of heterozygosity in natural populations. These markers have proved useful in the identification of the *Medicago* spp. and their hybrids. For example, the species *M. turbinata* (L.) All. and *M. truncatula* Gaertn. have different alleles at three loci, while *M. soleirolii* and *M. tornata* can be distinguished from each other on the basis of at least one locus (55,57). Furthermore, *M. sativa* ssp. *falcata* has a few specific allozymes not found in the closely related *M. sativa* ssp. *sativa* (L.) L.&L. and *M. sativa* ssp. *coerulea* Schmalh. (57). It is likely that with the development of additional enzyme systems in *Medicago*, the number of criteria to separate species will increase substantially. Another powerful tool that is becoming available to taxonomist involves the analysis of DNA restriction fragment length polymorphisms (RFLP analysis). This technique may prove to be more sensitive than the isozymes due to its ability to detect variation directly at the DNA level (5).

3-5.1.2 Key to *Medicago* Species

The general key constructed by Lesins and Lesins (40) is the best available guide for the identification of the *Medicago* spp. Their key is presented for reference purposes, together with a few modifications, mostly additions.

1	Perennials	2
—	Annuals; yellow flowers	25
2	Shrubs	<i>M. arborea</i> L.
—	Herbs	3
3	Corolla yellow	4
—	Corolla anthocyanin-colored; Violet, or variegated (i.e., all shades between violet and yellow)	23
4	Pods small (<3.5 mm long), kidney-shaped nutlets, containing one seed. Florets small (1-4 mm long)	<i>M. lupulina</i> L.
—	Pods and florets larger	5

ORIGIN AND EVOLUTION OF <i>MEDICAGO</i>		99
5	Pods straight or sickle-shaped, coiled in not more than one half-circle	6
—	Pods coiled in more than one half-circle	10
6	Pods narrow, 1 to 3 mm in width	<i>M. sativa</i> ssp. <i>falcata</i> Arcengeli
—	Pods more than 3 mm in width	7
7	Radicles and cotyledons of seeds with their long axes almost at right angles to pod's ventral suture	8
—	Radicles and cotyledons of seeds with their long axes almost parallel to the ventral suture	<i>M. hybrida</i> Trautv.
8	Pods 14 to 22 mm long, elliptical leaflets	<i>M. platycarpa</i> Trautv.
—	Pods 8 to 12 mm long	9
9	Pods wide, length <1.5 times width, florets with wings larger than the keel.	<i>M. cretacea</i> M.B.
—	Pods elongated, length two or more times width, florets with wings almost as large as the standard	<i>M. ruthenica</i> Ledeb.
10	Pods with spines or tubercles	11
—	Pods without spines or tubercles	15
11	Plants with rhizomes. Spines uniformly thick, 3 to 7 mm long. Seed with radicle at 45° angle to pod's ventral suture	<i>M. carstiensis</i> Wulf.
—	Plants without rhizomes. Spines thick at the base, short and rigid, or tubercles only. Seed with radicle almost parallel to the ventral suture	12
12	Pods and whole plants covered thickly with felted hairs; seashore plants	<i>M. marina</i> L.
—	Pods and plants not covered with felted hairs; not seashore plants	13
13	Pods 5 to 7 mm in diameter	14

100		QUIROS & BAUCHAN
—	Pods <5 mm in diameter. Usually entire, awl-shaped stipules.	<i>M. rhodopea</i> Velen.
14	Pods with glandular, articulated hairs. Standard oblong with sides parallel in the middle part $2n=16$.	<i>M. pironeae</i> Vis.
—	Pods without glandular, articulated hairs. Standard oval $2n=48$	<i>M. saxatilis</i> M.B.
15	Pod surface corrugated by a net of elevated veins	16
—	Pod surface smooth (after removal of hairs), or with only slightly elevated veins	17
16	Pods with one to three coils, 4 to 6 mm in diameter. $2n=48$, often narrow leaflets	<i>M. cancellata</i> M.B.
—	Pods with 1 to 1.5 coils, 3 to 4 mm in diameter. $2n=16$.	<i>M. rupestris</i> M.B.
17	Pods covered with articulate, semitransparent, as if membranous, glandular hairs. $2n=16, 32$.	<i>M. papillosa</i> Boiss.
—	Pod surface without glandular hairs. $2n=16, 2n=32$.	<i>M. dzhawakhetica</i> Bordz.
18	Leaflets nearly round, broadly ovate to obovate even at upper nodes, (length/width, 3:2), upper side of leaflets glabrous. Pollen grains triangular-pyramid-shaped.	<i>M. suffruticosa</i> Raymond
—	Leaflets oblong, narrowly ovate, at least at upper nodes, (length/width, more than 2:1); upper side of leaflets always somewhat hairy. Pollen grains spindle-cylindrical	19
19	Pods small, 2 to 4 mm in diameter, with or without glandular hairs	<i>M. prostrata</i> Jacq.
—	Pods larger, 5 to 9 mm in diameter, covered with glandular hairs	20

20	Pods large, 8 to 9 mm in diameter, with one to two loose coils, open in the center. In the keel and in the middle of the standard the yellow color is often of different intensity from the rest of the corolla.	<i>M. sativa</i> ssp. <i>glutinosa</i> M.B.
—	Pods smaller, 5 to 8 mm in diameter, coiled in 1.5 to 4 tight coils with only a small opening in the center. Corolla color uniform.	<i>M. glomerata</i> Balb.
21	Pods with spines	<i>M. daghestanica</i> Rupr.
—	Pods without spines	22
22	Corolla violet; standard and petals with parallel sides. Pods coiled in 1.5 to 4.0 tight coils, 2 to 5 mm diam, $2n=16$.	<i>M. sativa</i> ssp. <i>coerulea</i> Schmalh.
—	Flowers variegated, standard petals oval, pods coiled in 0.5 to 2.5 loose coils $2n=16, 32$	<i>M. sativa</i> ssp. \times <i>varia</i> Martin, <i>M. sativa</i> ssp. \times <i>hemicycla</i> Grossh.
23	Corolla violet, standard petals with parallel sides, pods coiled in two to five tight coils, 5 to 9 mm diam, $2n=32$	<i>M. sativa</i> ssp. <i>sativa</i> (L.) L.&L.
—	Flowers variegated, standard petals oval.	24
24	Pods with two to five coils, with a large opening in the center, covered with glandular hairs	<i>M. sativa</i> ssp. \times <i>tunetana</i> Murbeck
—	Pods twisted with one to two coils glandular hairs, with a small opening in the center, covered with glandular hairs	<i>M. polychroa</i> Grossh.
25	Veins on face of pod running obliquely from pod's ventral suture, do not change direction before joining the dorsal suture. Pods small, one-seeded nutlets. Pollen grains spindle-cylindrical	<i>M. lupulina</i> L.
—	Veins on face of pod change direction before joining the dorsal suture. Pollen grains triangular pyramid-shaped	<i>M. secundiflora</i> Durieu

26	Seeds with long axes almost at right angle to pod's ventral suture; seedcoats ridged or verrucose, pods larger than 3.5 mm long with at least one-fourth coils	27
—	Seeds with long axes almost parallel to the ventral suture; seedcoats smooth	29
27	Pods spiny. Seedcoats ridged	28
—	Pods flat and spineless. Seedcoats verrucose	<i>M. orbicularis</i> Bart.
28	Coils with one row of spines, coil edge paper-thin	<i>M. radiata</i> L.
—	Coils with two rows of spines, coil edge wide.	<i>M. heyniana</i> Greuter
29	Seeds black or red brown	30
—	Seeds yellow or yellow brown	33
30	Pods large, with 8 to 10 coils, florets large, 8 to 10 mm long; seeds large, 13 to 17 g/1000	31
—	Pods smaller, with five to seven coils; florets smaller, 5 to 7 mm long, seeds smaller, 7.5 to 10 g/1000	32
31	Pods and spines with glandular, articulated hairs	<i>M. ciliaris</i> All.
—	Pods glabrous or with a few simple hairs, leaflets often with anthocyanin spot	<i>M. intertexta</i> Mill.
32	Pods cylindrical or disk-shaped with truncate apex and base	<i>M. muricoleptis</i> Tineo
—	Pods more round, barrel-shaped	<i>M. granadensis</i> Willd.
33	Pods densely covered with long hairs, resembling small cotton balls	<i>M. lanigera</i> Winkl. & Fedtsch.
—	Pods without long hairs	34
34	Coils imbricate like a set of bowls, with their convex parts towards apex and base, or towards base only	35
—	Coils not markedly imbricate	36

35	Coils with their convex parts towards pod base only. $2n=30$	<i>M. scutellata</i> Mill.	
—	Coils with their convex parts towards both base and apex $2n=16$	<i>M. blancheana</i> Boiss.	
36	Coil edges with ridges, or with wing-like elevations running obliquely or at right angle to the dorsal suture		37
—	Coil edges smooth, or spined, or with tubercles		39
37	Wing-like elevations on coil edges running at right angle to pod's dorsal suture. Pods small, 3 to 5 mm in diameter, usually with 1.5 coils	<i>M. shepardii</i> Post	
—	Ridges on coil edges running obliquely towards the dorsal suture. Pods more than 5 mm in diameter with 2.5 to 5 coils		38
38	Dorsal suture usually in a groove in the middle of the coil edge. Calyx appressed to the base of the pod as a regular star. $2n=16$	<i>M. noeana</i> Boiss.	
—	Dorsal suture elevated in the middle of the coil edge. Calyx appressed sideways to the base of the pod. $2n=30$	<i>M. rugosa</i> Desr.	
39	Pollen grains shaped like irregular blocks, pods cylindrical, spines short (0.5–2 mm), inserted in the margins of the coil edges almost at right angle to the face of the coil; apical coil concave. Leaflets often notched and with anthocyanin basal fleck.	<i>M. rotata</i> Boiss.	
—	Pollen grains spindle-cylinder, or triangular pyramid-shaped.		40
40	Pods soft-walled. Central part of each coil consisting mainly of veins with thin membranous tissue between them (coils may be pulled apart releasing the		

	seed). Spines, if present, slender, their base with two prongs (roots) connected by a membrane, one prong inserted in the dorsal suture, the other in the lateral vein or in a veinless zone	41
—	Pods hard-walled (for release of seed, crushing of pod may be necessary). Spines, if present, stocky, their base conical, often embedded in spongy tissue. Venation on the face of the coil usually not clearly discernible.	49
41	Face of coil with radial veins running into a veinless zone	42
—	Face of coil with radial veins running into a lateral vein	43
42	Pod edge grooved; spines slanted away from the apical coil; apical coil spineless	<i>M. disciformis</i> DC.
—	Pod edge level; spines pointing to both apical and basal end	<i>M. tenoreana</i> Ser.
43	Pod edge level or slightly concave, completely or almost completely covering grooves between the edge and lateral veins	44
—	Pod edge grooved, grooves between dorsal suture and lateral veins observable in edge on view	45
44	Peduncle usually many-flowered (up to 17 florets). $2n=16$	<i>M. coronata</i> Bart.
—	Peduncle few-flowered (1-2 florets). $2n=14$	<i>M. praecox</i> DC.
45	Dorsal suture of pod lying in a groove; on pod edge alternate four ridges with three grooves. Leaflets usually with dark spot	<i>M. arabica</i> Huds.
—	Dorsal suture of pod elevated above lateral veins.	46

46	Lateral veins on face of coil at one-third to two-fifths of the radius below the dorsal suture. Plants densely hairy. Stipules entire or slightly toothed.	<i>M. minima</i> Bart.
—	Lateral veins on face of coil at one-third or less of the radius below the dorsal suture. Stipules deeply incised	47
47	Florets with wings longer than the keel. $2n=14$	<i>M. polymorpha</i> L.
—	Florets with wings shorter than the keel. $2n=16$	48
48	Apical coil spiny; lateral veins on coil face joining as shoulders at right angle to the elevated dorsal suture	<i>M. laciniata</i> Mill.
—	Apical coil spineless; lateral veins only slightly protruding from the coil face	<i>M. sauvagei</i> Nègre
49	Coils spineless; coil face without lateral vein or veinless zone; coils tightly appressed	<i>M. soleirolii</i> Duby
—	Coil face with lateral veins or veinless zone	50
50	Radial veins ending in a veinless zone on coil face	51
—	Radial veins ending in a lateral vein on coil face	53
51	Veinless zone wide, about one-third of coil radius; upper side of leaves completely glabrous	52
—	Veinless zone narrower, one-fourth to one-fifth of coil radius, upper side of leaves at least sparsely hairy	<i>M. turbinata</i> All.
52	Leaflets with small whitish patches, longer and thinner stems, edge of pod coils with none to three ridges, $2n=16$	<i>M. lesinsii</i> Small
—	Leaflets without patches, shorter and thicker stems, edge of pod coil with three ridges, $2n=14$.	<i>M. murex</i> Willd.

106		QUIROS & BAUCHAN
53	Pods convex at both ends, subspherical or oval in shape. Young pod contracted and concealed within calyx.	<i>M. doliata</i> Carmign.
—	Pod ends truncate, pods cylindrical or subcylindrical	54
54	Coils of pod tightly appressed, with no slits between them in dry mature pods; juncture between individual coil edges not markedly depressed	55
—	A continuous or interrupted slit between coil in dry mature pods; coil edges usually sloped towards their juncture	57
55	No groove on pod edge between dorsal suture and lateral veins. Radial veins on pod face strongly curved, running almost concentric before joining the lateral vein. $2n=14$	<i>M. constricta</i> Dur.
—	On edge of immature pods a shallow groove between dorsal suture and lateral veins, disappearing at pod maturity. Radial veins only slightly curved. $2n=16$	56
56	Pods glabrous; dorsal suture usually not higher than margins of the edge; spines, if present, inserted at 180° to the plant of coil face or obliquely to it.	<i>M. littoralis</i> Rohde
—	Pods with sparse hairs; dorsal suture usually strongly protruding in the middle of coil edge; spines inserted at 90° or obliquely to the coil face	<i>M. truncatula</i> Gaertn.
57	Dorsal suture in the middle of an evenly convex pod edge. Radial veins somewhat curved. $2n=16$	<i>M. tornata</i> Mill.
—	Shallow groove between dorsal suture and lateral veins that disappears at pod maturity. Radial veins strongly curved. $2n=14$.	<i>M. rigidula</i> (L.) All.

3-6 THE *MEDICAGO SATIVA* COMPLEX

3-6.1 Species Forming the Complex

The taxa constituting the *M. sativa* complex belong to the section *Falcago*, subsection *Falcatae* within the genus *Medicago*, which includes diploid and/or tetraploid forms of the species, *M. sativa* ssp. *sativa*, *M. sativa* ssp. *falcata*, and *M. sativa* ssp. *glutinosa*. They intercross with each other and share the same karyotypes (14). These taxa have been given the hierarchy of species by some authors (40) and of subspecies by others (15). Considering that the main barrier for gene exchange among them is ploidy, which might be often broken by diploids generating unreduced gametes (46,68), it is justifiable to classify them as subspecies, especially when no hybridization barriers are present and where genetic evidence supports common ancestry. Other species closely related to the *M. sativa* complex forming part of the same gene pool are *M. glomerata* and *M. prostrata*.

On the basis of legume and flower characters, Gunn et al. (15) considered all taxa within the complex as subspecies of a single species, *M. sativa*. They recognized a total of nine subspecies. Some of these are questionable, however, because the range of variation for some of the traits used to separate the taxa, either overlap or did not agree with previous descriptions. For example, their descriptions for *M. sativa* ssp. \times *varia* Arcangeli and *M. sativa* ssp. *ambigua* Tutin overlap for corolla color, being differentiated mainly on the basis of pilosity, pod shape, flower size; C-shaped to spiraled pilose for the former and, glabrous, falcate pod shape for the latter. Furthermore, Gunn et al. (15) recognized *M. sativa* ssp. *ambigua* as a diploid form. They apparently chose the name *ambigua* over *trautvetteri*, an epithet commonly used to describe this particular taxa, reported to occur in diploid and tetraploids forms (40). Gunn et al. (15) described *M. sativa* ssp. *glomerata* (Balbis) Tutin as lacking glandular hairs in the legume. However, Lesins and Lesins (40), Ivanov (20), Lubenets (44), and Sinskaya (66) among others, agreed in their description of *M. glomerata* as a species characterized by legumes covered with glandular hairs. Furthermore, changing the status of *M. glomerata* to *M. sativa* ssp. *glomerata* is unjustifiable because of the incipient genetic barrier, reflected in abnormal meiocytes and low fertility in F₁ hybrids between *M. sativa* and *M. glomerata* (26). Gunn et al. (15) apparently renamed *M. glutinosa*, a tetraploid with yellow flowers and coiled pods as *M. sativa* ssp. *praefalcata* Sinsk., and included under this taxa diploid and tetraploid accessions. The subspecies name *praefalcata* is misleading because it implies that it is the ancestor of *M. sativa* ssp. *falcata*.

The morphological variability in species of the *M. sativa* complex

accounts for the proliferation of epithets to name them. In many situations, only subtle morphological differences which might have been the product of genetic recombination, have been accepted as sufficient evidence to create a new species or subspecies. With respect to this problem, Lesins and Gillies (35) wrote: "A most confusing situation arises when taxa interbreed freely and the offspring have unimpaired viability. On recombination of parental characters, hybrid swarms are produced covering the range of variability between the parental species. This is the situation with the *M. sativa*, *M. falcata*, and *M. glutinosa*." Evidence for this is the large number of separate species names assigned to hybrids which include more than 50 described species and close to 100 names below the species rank. Table 3-1, adapted from Lesins and Lesins (40) lists most of the epithets for each of the species in the complex and their hybrids. On the basis of the present taxogenetic evidence, eight subspecies

Table 3-1. Species epithets used for subspecies of the *Medicago sativa* complex. Adapted from Lesins and Lesins (39).

ssp. <i>sativa</i> , ssp. <i>coerulea</i>	ssp. <i>falcata</i>	ssp. <i>glutinosa</i>	<i>M. glomerata</i>
<i>afghanica</i> Vass.	<i>altissima</i> Grossh.	<i>gunibica</i> Vass.	<i>sativa</i> ssp. <i>glomerata</i> Rouy
<i>agropyretorum</i> Vass.	<i>borealis</i> Grossh.		<i>sativa</i> ssp. <i>faurei</i> Maire
<i>asiatica</i> Sinsk.	<i>difalcata</i> Sinsk.		
<i>asiatica</i> Sinsk.	<i>difalcata</i> Sinsk.		
<i>coerulea</i> Gunn et al.	<i>erecta</i> Kotov		
<i>coerulea</i> Less.	<i>glandulosa</i> David.		
<i>jemenensis</i> Sinsk.	<i>quasifalcata</i> Sinsk.		
<i>kopetdaghi</i> Vass.	<i>romanica</i> Prod.		
<i>laurenkoi</i> Vass.	<i>tenderensis</i> Opperm.		
<i>mesopotamica</i> Vass.			
<i>orientalis</i> Vass.			
<i>polia</i> Vass.			
<i>sogdiana</i> Vass.			
<i>subdicycla</i> Vass.			
<i>tadzhicorum</i> Vass.			
<i>transoxana</i> Vass.			
ssp. × <i>hemicycla</i> , ssp. × <i>varia</i> : Hybrids between ssp. <i>sativa</i> or ssp. <i>coerulea</i> and ssp. <i>falcata</i>		ssp. × <i>polychroa</i> : Hybrids between ssp. <i>sativa</i> and ssp. <i>glutinosa</i>	ssp. × <i>tunetana</i> : Hybrids between ssp. <i>sativa</i> or ssp. <i>coerulea</i> and <i>M. glomerata</i>
<i>alaschanica</i> Vass.	<i>laurenkoi</i> Vass.	<i>glutinosa</i> M.B.	<i>tunetana</i> Vass.
<i>alata</i> Vass.	<i>media</i> Pers.	<i>grossheimii</i> Vass.	
<i>beipinensis</i> Vass.	<i>ochroleuca</i> M. Kult.	<i>polychroa</i> Grossh.	
<i>caucasica</i> Vass.	<i>roborovskii</i> Vass.	<i>virescens</i> Grossh.	
<i>falcata</i> L.	<i>rivularis</i> Vass.		
<i>gaetula</i> Trbut.	<i>sativa</i> L.		
<i>grandiflora</i> Vass.	<i>schischkinii</i> Sumn.		
<i>hemicoerulea</i> Sinsk.	<i>tianschanica</i> Vass.		
<i>hemicycla</i> Grossh.	<i>tibetana</i> Vass.		
<i>komarovii</i> Vass.	<i>trautvetteri</i> Sumn.		
<i>kultiassovii</i> Vass.	<i>vardanis</i> Vass.		
<i>ladak</i> Vass.	<i>varia</i> Mart.		

inated from tetraploidized hybrids of the diploids *M. sativa* ssp. *coerulea* and *M. glomerata* (40). *Medicago sativa* ssp. \times *polychroa* Grossh. has been described as a tetraploid, which seems to have originated from the hybridization of the tetraploid *M. sativa* ssp. *sativa* and *M. sativa* ssp. *glutinosa* (40). These two hybrid subspecies have been studied very little, and their classification is tentative. The other two subspecies, \times *varia* and \times *hemicycla* may have resulted from the hybridization of *M. sativa* ssp. *falcata* with *M. sativa* ssp. *sativa* and *M. sativa* ssp. *coerulea*, respectively. The former is tetraploid and the latter diploid.

Medicago glomerata is a species related to the *M. sativa* complex, characterized by bright yellow flowers and coiled pods covered with glandular hairs. It is found in the diploid form in southern Europe, Mountain Alps, and North Africa. In North Africa, however, a tetraploid species described as *M. sativa* ssp. *faurei* by Moire (40) has been considered on morphological grounds, as the tetraploid form of *M. glomerata*. *Medicago glomerata* is more closely related to the species *M. prostrata* than to taxa of the *M. sativa* complex (26).

Medicago prostrata, a species found in diploid and tetraploid forms, is characterized also by yellow flowers and coiled pods. Its pods are similar to those of *M. sativa* ssp. *coerulea*, and has yellow flowers in common with *M. sativa* ssp. *falcata*. Inflorescences of *M. prostrata* are less dense and have fewer florets, however, than those of *M. sativa* (35). *Medicago prostrata* crosses with the species of the *M. sativa* complex when it is used as the pollen parent. In reciprocal crosses, only poorly developed seeds are produced, thus a postzygotic barrier seems to exist between these species (25).

3-6.2 Evolutionary Trends of the *M. sativa* Complex

Much speculation is involved in the events that lead to the morphological differentiation of the diploid *M. sativa* ssp. *coerulea* and *M. sativa* ssp. *falcata*. Lesins and Lesins (38) proposed that diploid *M. glomerata*, a species characterized by yellow flowers and coiled pods, was once distributed farther to the east colonizing a large territory spreading to the Caucasus where it served as the probable ancestor of the diploids in the complex. It is projected as giving rise to both subspecies, *coerulea* and *falcata*, by spatial isolation of the ancestral population which was divided by Parathethys, (they connect Black and Caspian Seas), during the Tertiary Period. The southern population resulting from this division, ssp. *coerulea*, suffered a loss of carotenoids and again in anthocyanin in their flowers, due to selection pressures imposed by competition for pollinators in the newly isolated area. The northern population, ssp. *falcata*, involved selective pressure for straight pods, thus shatter for easy seed dispersal. This characteristic might have been favored in the north, where thick grasslands would prevent the dispersal of coiled pods, adapted to rolling on open ground. The change must have been gradual, because pod shape

bution of diploid *M. sativa* ssp. *falcata* over a wide geographical range, the great distances between existing populations, and the extensive variability observed in this subspecies, indicate that it was widespread at one time over a large territory. The distribution pattern might result from glaciation during the ice age in Pleistocene. During this geological event, only those populations occupying unglaciated sites were able to survive (38).

Recent evidence demonstrating the occurrence of unreduced gametes (46,53,78) in diploid subspecies of *M. sativa*, indicates that this might have been a significant event in the origin of the tetraploid subspecies. Previous reports had indicated the presence of a ploidy barrier between diploid and tetraploids, preventing intercrossing in nature. These studies were based on crosses among a limited number of diploid and tetraploid accessions. In any event, $2x-4x$ or $4x-2x$ crosses are possible when the diploid parent has the ability to generate unreduced ($2n$) gametes, resulting in tetraploid progeny. Spontaneous chromosome doubling cannot be discounted in the generational tetraploids, although it seems to be more the exception than the rule in the evolution of polyploid species (16). The hypothetical evolution of species of the *M. sativa* complex is summarized in Fig. 3-3 on the basis of available data.

3-6.2.1 Diploids

Quiros and Morgan (56) reported the inheritance of several isozymes in diploid *M. sativa* ssp. *falcata*, *M. sativa* ssp. *coerulea*, and *M. sativa* ssp. \times *hemicycla* Grossh. On the basis of these genetic markers, the genetic variability of natural diploid populations was summarized by Quiros (55). Results obtained for each of these three diploid subspecies and for *M. glomerata* for four polymorphic loci, *Prx-1*, *Prx-2*, *Lap-1*, and *Lap-2* coding for peroxidase (PRX) and leucine amino peptidase (LAP) isozymes are shown in Table 3-2. Thirteen accessions of *M. sativa* ssp. *falcata* and seven accessions of *M. sativa* ssp. *coerulea* were sampled from the entire range of distribution of the species. Only three accessions of *M. sativa* ssp. \times *hemicycla*, two from Armenia and one from the Transcaucasus, and three accessions of *M. glomerata*, all from Italy, were included in the study. In agreement with the morphological data, *M.*

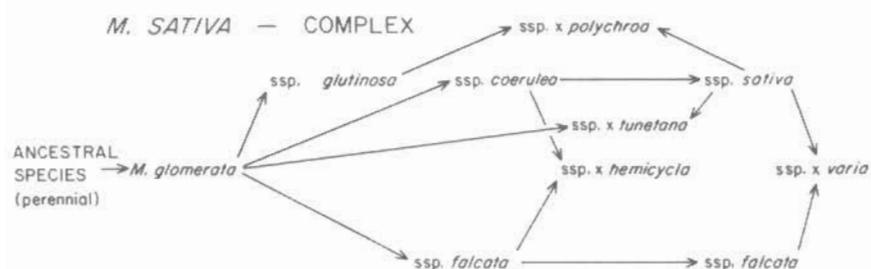


Fig. 3-3. Possible evolutionary pathway of the *M. sativa* complex and closely related species.

Table 3-2. Genetic variability of four polymorphic isozyme loci *Prx-1*, *Prx-2*, *Lap-1*, and *Lap-2* found in diploid and tetraploid subspecies of the *Medicago sativa*-complex and in *M. glomerata*.†

Ploidy	Species	No. accessions	No. plants	No. of alleles		Percentage heterozygosity
				\bar{x}	Range	
Diploid	<i>M. glomerata</i>	3	95	8.3	8-9	16.7
Diploid	<i>M. sativa</i> ssp. <i>coerulea</i>	7	368	8.6	5-10	20.5
Diploid	<i>M. sativa</i> ssp. <i>falcata</i>	13	447	12.0	7-17	27.7
Diploid	<i>M. sativa</i> ssp. \times <i>hemicycla</i>	3	190	16.3	14-20	50.9
Tetraploid	<i>M. sativa</i> ssp. <i>sativa</i>	6	173	9.8	9-14	37.5
Tetraploid	<i>M. sativa</i> ssp. \times <i>varia</i>	5	165	11.2	9-14	49.2
Tetraploid	<i>M. sativa</i> ssp. <i>falcata</i>	10	378	14.1	10-20	57.92
Tetraploid	Cultivated alfalfa	17	776	10.9	7-16	34.72

† Prx = peroxidase, Lap = leucine amino peptidase.

sativa ssp. *falcata* was found to be significantly more variable than *M. sativa* ssp. *coerulea*, measured by the number of alleles and the percentage of heterozygous individuals in the population. Although the sample of *M. sativa* ssp. \times *hemicycla* was small, its variability measured by both criteria vastly surpassed that of *M. sativa* ssp. *falcata*. This was consistent for the three accessions of *M. sativa* ssp. \times *hemicycla* sampled. Only one accession of *M. sativa* ssp. *falcata* (UAG 127 from Bulgaria) carrying a total of 17 alleles and 47.75% heterozygosity came close to the values obtained for *M. sativa* ssp. \times *hemicycla*. On the other hand, the degree of genetic variability for *M. glomerata* was considerably lower. Although these data are not extensive enough to support solid conclusions on the evolution of the *M. sativa* complex, they provide further evidence on the origin of the species. According to Sinskaya (66), *M. sativa* ssp. \times *hemicycla* is the ancestor of both *M. sativa* ssp. *falcata* and *M. sativa* ssp. *coerulea*. Lesins and Lesins (40) and Ivanov (20) argued instead that *M. sativa* ssp. *hemicycla* is of recent origin, being the product of hybridization between *M. sativa* ssp. *falcata* and *M. sativa* ssp. *coerulea*. They based their conclusion in the fact that artificial hybrids between both species fall within the spectrum of flower color and pod coiling of *M. sativa* ssp. \times *hemicycla* and in the present range of distribution of the three species (40). This natural hybridization might have taken place at more than one location, where the two subspecies were in contact. The intermixing of two gene pools would increase the variability of the hybrid subspecies.

The electrophoretic data demonstrates that *M. sativa* ssp. \times *hemicycla* includes accessions that are the most variable of all the taxa tested. It shares specific allozymes with *M. sativa* ssp. *falcata* which are not found in other subspecies of *M. sativa* (55). Thus, it is conceivable that *M. sativa* ssp. \times *hemicycla* has resulted from the hybridization of *M. sativa* ssp. *coerulea* and *M. sativa* ssp. *falcata*. Sinskaya's hypothesis, however, cannot be discounted until additional accessions and loci are sampled.

3-6.2.2 Tetraploids

Tetraploid subspecies in the *M. sativa* complex, *M. sativa* ssp. *sativa*, *M. sativa* ssp. \times *varia*, *M. sativa* ssp. *falcata*, and *M. sativa* ssp. *glutinosa* are distinguished from their diploid counterparts by the larger size of their flowers, pods and seeds. *Medicago sativa* ssp. *varia* arose by hybridization of *M. sativa* ssp. *sativa* with *M. sativa* ssp. *falcata* (40) which still occurs in the numerous locations where the two subspecies are sympatric. This takes place at diploid, tetraploid, and most likely at diploid-tetraploid levels with unreduced gametes generated by diploid accessions (46,51,53,78). The numerous epithets given to *M. sativa* ssp. \times *varia* are listed in Table 3-1.

Stanford (76) provided conclusive evidence of autotetraploidy in alfalfa, after finding tetrasomic segregation for flower color in progenies of diallelic plants. Quiros (54) proved the existence of multiple alleles for several isozyme loci. On the basis of these genetic markers tetrasomic segregation in di-, tri-, and tetraallelic plants has been documented in alfalfa. In that study, it was observed that segregating progenies of *sativa-falcata* hybrids did not deviate from expected ratios, supporting their classification as subspecies of a common species.

Together with data obtained from diploid subspecies, Quiros (55) surveyed the genetic variability of both commercial cultivars of alfalfa from diverse origins, and of "natural" tetraploids obtained from the entire range of distribution of *M. sativa*. These "natural" tetraploids included feral collections and primitive cultigens of the *M. sativa* ssp. *sativa*, *M. sativa* ssp. \times *varia* (forms *tianschianica* and *hemicoerulea*) and *M. sativa* ssp. *falcata*. They were collected by the later professor Karl Lesins, forming part of the *Medicago* collection housed at the Univ. of Alberta. Commercial alfalfa was represented by 16 cultivars (Table 3-4), from seven different sources, as identified by Barnes et al. (2). In general, natural tetraploids were found to be more variable than their diploid counterparts, measured by percentage heterozygosity (Table 3-2). This is expected, because the former have four copies for each chromosome. Tetraploid *M. sativa* ssp. *falcata* was found to have the highest values for

Table 3-3. Number of tetraallelic plants found in alfalfa cultivars and in tetraploid accessions of *Medicago sativa* ssp. *falcata* from various origins (55).

Cultivar/Accession	No. of plants	Tetraallelic plants for:		
		Prx-1†	Lap-2‡	Percentage
Hairy Peruvian	25	4	0	16.0
Sonora	106	3	0	2.8
Leningrad (UAG 110)	46	2	0	4.3
Czechoslovakia (UAG 102)	70	3	0	4.3
Latvia (UAG 105)	51	4	0	7.8
Siberia (UAG 113)	48	4	9	27.1
Turkey (UAG 2008)	18	0	6	33.3

† Prx = peroxidase.

‡ Lap = leucine amino peptidase.

Table 3-4. Characteristic isozymes present in various cultivars of different origins (55).

Origin	Cultivar	Allozymes				Subspecies involved according to: Quiros		
		PRX†		LAP‡		Barnes et al (2)	Quiros (55)	
Turkistan	Deseret Lahontan Nemastan	1 ³	2 ³ 2 ³	2 ⁿ 3 ³ 2 ⁿ 3 ³ 3 ³	2 ²	Mostly <i>sativa</i>	<i>sativa</i>	
Indian	Mesa Sirsa	1 ⁸	2 ³	3 ³	2 ² 2 ³	Only <i>sativa</i>	<i>sativa</i>	
Peruvian	Hairy Peruvian Monsefu				2 ³ 2 ² 2 ³	Only <i>sativa</i>	<i>sativa</i>	
Ladak	Ladak		1 ⁿ 2 ⁿ		2 ³	Mostly <i>sativa</i>	<i>sativa</i> and <i>falcata</i>	
Flemish	Tuna Du Puits Socheville Europe	1 ³ 1 ⁶ 1 ⁸ 1 ³ 1 ⁸ 2 ³ 1 ³ 1 ³	2 ³ 2 ³	3 ³ 3 ³	2 ² 2 ³ 1 ³ 2 ² 2 ³ 2 ⁴ 2 ⁵ 1 ³ 2 ² 2 ³	Only <i>sativa</i>	<i>sativa</i> and <i>falcata</i>	
African	A-435 Sonora Moapa		2 ³ 2 ³ 2 ⁵ 2 ³	3 ³ 3 ³	1 ⁵ 2 ²	2 ⁵ 2 ⁶	Unre- ported	<i>sativa</i> and <i>falcata</i>
Chilean	Buffalo Chilean		2 ³	1 ² 3 ³		2 ⁶ 2 ⁶	Only <i>sativa</i>	<i>sativa</i> and <i>falcata</i>

† PRX = peroxidase.

‡ LAP = leucine amino peptidase.

both, percentage heterozygosity and number of alleles, among all subspecies including both diploids and tetraploids. Interestingly, the percentage heterozygosity for the *M. sativa* ssp. × *hemicycla*, a diploid, was similar to that of the hybrid tetraploid *M. sativa* ssp. × *varia*. This can be explained by the fact that the former, on the average, has twice as many alleles as the latter. The level of variability of the cultivated alfalfa, which included cultivars known to carry *M. sativa* ssp. *falcata* genes, was comparable to that observed for the natural populations of *M. sativa* ssp. *sativa* but far below the values obtained for *M. sativa* ssp. *falcata* and *M. sativa* ssp. × *varia*. When the percent heterozygosity in the tetraploid populations was reduced to the level of individual plant heterozygosity, it was found that there were twice as many tri- and tetraallelic plants in the natural tetraploids as in the cultivars. In either case, the average frequency of tetraallelic plants was <1.5%, while that of triallelic plants was 9% for the cultivars and 19% for the natural tetraploids. Table 3-3 lists the accessions of natural tetraploids and cultivars where tetraallelic plants were found for the two most polymorphic loci. Tetraallelic plants in the natural tetraploids were found only in *M. sativa* ssp. *falcata*. In particular, two accessions, one from Siberia, another from Turkey and the cv. Hairy Peruvian, were the richest in tetraallelic plants, considering

the small expected probability of drawing tetraallelic plants from random populations (6,10). These results support the hypothesis that maximum heterozygosity, reflected in tri- and tetraallelic plants, plays an important adaptive role in autotetraploids (6).

3-6.2.3 Origin of Cultivated Alfalfa

According to Lesins (31), the hybridization of the *M. sativa* ssp. *sativa* and *M. sativa* ssp. *falcata* might have contributed to the cultivation of alfalfa throughout much of the temperate zone.

Ivanov (20) suggests that alfalfa was cultivated 8000 to 9000 yr ago. The most likely centers for alfalfa domestication are the Armenia Highland, including the trans-Caucasus, Turkey, and Iran; and Southern Turkistan. Its domestication was perhaps concurrent with that of the horse (31). From the original centers of cultivation, alfalfa spread to Mesopotamia, the Old World, People's Republic of China, and India. In the 16th century, it was introduced to Mexico and Peru by the Spanish. It reached North America after several introductions in the 1800s from Mexico by the missionaries, and from Chile to California, as "Chilean clover" during the gold rush.

Barnes et al. (2) summarized the sources of alfalfa introductions to North America. They gave an account of the subspecies of *M. sativa* contributing to each of these germplasm sources, on the basis of historical information and morphological and physiological attributes. Specific isozymes can be used to differentiate *M. sativa* ssp. *falcata* from *M. sativa* ssp. *sativa* and to trace the ancestors of alfalfa cultivars (55). The alleles *Prx-1⁷*, *Prx-1¹⁵*, *Prx-1ⁿ*, *Prx-2⁵*, *Lap-1⁵*, *Lap-2⁴*, *Lap-2⁵*, and *Lap-2⁶*, have been found only in *M. sativa* ssp. *falcata* and in the hybrid ssp. \times *hemicycla* and \times *varia*. Many other isozymes are common to all subspecies of the *M. sativa* complex. Thus, it is possible to determine if ssp. *falcata* genes are present in a given cultivar on the basis of the alleles listed above. Sixteen cultivars of diverse origins were tested (Table 3-4). Good agreement was found between the isozyme data and the conclusions reached by Barnes et al. (2). The only exceptions were the cultivars of Flemish and Chilean origins, which are assumed to have only ssp. *sativa* genes. The isozyme data indicates that *M. sativa* ssp. *falcata* is also involved in their genetic makeup. In addition, it was found that *M. sativa* ssp. *falcata* had contributed to the development of African alfalfas. In general, cultivars of the same origin shared the same alleles. A few cultivars could be distinguished by rare alleles, such as A-435 (African) by allele *Lap-1¹⁵*, and Ladak by the null allele *Prx-1ⁿ*. Several alleles found in diploid and tetraploid *M. sativa* ssp. *falcata* accessions from northern latitudes were not encountered in any of the cultivars tested. This could suggest the availability of germplasm which has not been exploited in alfalfa breeding. Conversely, this assessment is based on only two isozyme systems and additional data are needed.

3-7 TAXOGENETICS OF *MEDICAGO*

3-7.1 Hybridization and Crossing Relationships

The ability of species to be crossed with one another is perhaps the best indicator of their relationship. The crossing relationship of the subspecies in the *M. sativa* complex are described in the previous section of this chapter and in Chapter 24 in this book. Hybridization of species in the *M. sativa* complex with other wild perennial *Medicago* spp. is also reported in Chapter 24 in this book. A summary of the successful inter-specific crosses between *Medicago* spp. can be found in Table 3-6.

Little hybridization work has been done with the annual *Medicago* spp. Most of the taxonomic classification is based on morphological traits. Hybridization between *M. rotata* and *M. blanchiana* occurs frequently in nature. This is supported by the fact that the hybrids between these two species have been described as several subspecies (40).

Successful hybridizations between species in the section *Pachyspirae* (Table 3-5) have been limited because of the occurrence of chlorophyll-deficient plants and early embryo abortion. Crosses between *M. truncatula* (as *M. striata*) and *M. littoralis* are possible with good chromosome pairing in the hybrid. However, when *M. littoralis* is used as the maternal parent the resulting hybrids are chlorophyll deficient, with many of the plants occurring as chimeras. The reciprocal cross results in hybrid plants with normal green foliage and morphological characteristics intermediate between the parents (33,64). *Medicago tornata* can be hybridized with *M. littoralis* but the hybrid displays the same type of chlorophyll deficiency (33,63). *Medicago tornata* is more distantly related to *M. littoralis* than *M. truncatula* judged by the presence of a chromosomal translocation (63). Likewise, hybrids between *M. soleirolii* and *M. tornata* are chlorophyll deficient (40). Lesins et al. (32) were able to hybridize *M. turbinata* with *M. truncatula* only when *M. truncatula* was used as the maternal parent. The hybrids were sterile with lighter green foliage than either of the parents. Fertility was recovered when the chromosome number of the hybrid was doubled. Lack of segregation for several traits including isozymes in the hybrid and the occurrence of preferential pairing, indicates that these two species have different genomes (32,57).

The chlorophyll-deficiency syndrome was noted, as well, in hybrids between *M. laciniata* and *M. sauvagei* which is taxonomically in a different section (*Leptospirae*) (Table 3-5) (65). These two species are very closely related to each other as normal bivalent pairing occurs in their hybrid.

Medicago intertexta, *M. ciliaris*, and *M. muricoleptis* can be intercrossed with each other. *Medicago muricoleptis* is more distantly related to *M. intertexta* than *M. ciliaris* judged by the reduced pollen fertility and chromosome pairing observed in hybrids between *M. muricoleptis* and either *M. intertexta* or *M. ciliaris*. Some taxonomists consider *M.*

Table 3-5. *Medicago* spp., grouped by subgenus and sections.

Subgenus <i>Lupularia</i>	Subgenus <i>Spirocarpos</i>
<i>M. lupulina</i>	Section <i>Rotatae</i>
<i>M. secundiflora</i>	<i>M. rotata</i>
	<i>M. blancheana</i>
Subgenus <i>Orbicularia</i>	<i>M. noeana</i>
Section <i>Carstiensae</i>	<i>M. shepardii</i>
<i>M. carstiensis</i>	<i>M. rugosa</i>
Section <i>Platycarpae</i>	<i>M. scutellata</i>
<i>M. platycarpa</i>	Section <i>Pachyspirae</i>
<i>M. ruthenica</i>	<i>M. soleirolii</i>
Section <i>Orbiculares</i>	<i>M. tornata</i>
<i>M. orbicularis</i>	<i>M. littoralis</i>
Section <i>Hymenocarpos</i>	<i>M. truncatula</i>
<i>M. radiata</i>	<i>M. rigidula</i>
Section <i>Heyniana</i>	<i>M. murex</i>
<i>M. heyniana</i>	<i>M. lesnii</i>
Section <i>Cretaceae</i>	<i>M. constricta</i>
<i>M. cretacea</i>	<i>M. turbinata</i>
	<i>M. doliata</i>
Subgenus <i>Medicago</i>	Section <i>Leptospirae</i>
Section <i>Falcago</i>	<i>M. sauvagei</i>
Subsection <i>Falcatae</i>	<i>M. laciniata</i>
<i>M. sativa</i>	<i>M. minima</i>
<i>M. glomerata</i>	<i>M. praecox</i>
<i>M. prostrata</i>	<i>M. coronata</i>
Subsection <i>Rupestris</i>	<i>M. polymorpha</i>
<i>M. rhodopea</i>	<i>M. arabica</i>
<i>M. saxatillis</i>	<i>M. lanigera</i>
<i>M. rupestris</i>	<i>M. disciformis</i>
<i>M. cancellata</i>	<i>M. tenoreana</i>
Subsection <i>Daghnestanicae</i>	Section <i>Intertextae</i>
<i>M. daghestanica</i>	<i>M. intertexta</i>
<i>M. pironae</i>	<i>M. ciliaris</i>
Subsection <i>Papillosae</i>	<i>M. muricoleptis</i>
<i>M. dzhawakhetica</i>	<i>M. granadensis</i>
<i>M. papillosa</i>	
Section <i>Arborea</i>	
<i>M. arborea</i>	
Section <i>Marinae</i>	
<i>M. marina</i>	
Section <i>Suffruticosae</i>	
<i>M. suffruticosa</i>	
<i>M. hybrida</i>	

ciliaris to be a subspecies of *M. intertexta* (40). Refer to Table 3-6 for a summary of the successful hybridizations among the annual species.

The only hybrid which has been obtained between a perennial and an annual *Medicago* is that of *M. sativa* ssp. *sativa* × *M. scutellata* (59). Sangduen et al. (59) proposed that the *M. sativa* clone, a male sterile used as the female parent, generated unreduced eggs which united with normal haploid gametes from *M. scutellata* producing a mixaploid with the primary stem being hexaploid. The hybrid was both male and female sterile. The hybrid was perennial and purple flowered like the *M. sativa* parent.

Table 3-6. Successful interspecific hybridization between *Medicago* spp.†

Interspecific hybrids	References
Perennials	
<i>cancellata</i> × <i>sativa</i> & reciprocal	23, 74, 80
<i>cancellata</i> × <i>saxatilis</i>	28
<i>daghestanica</i> × <i>pironae</i>	34
F ₁ (<i>daghestanica</i> × <i>pironae</i>) × <i>sativa</i>	29
<i>dzhawakhetica</i> × <i>sativa</i> & reciprocal	8, 23, 39, 50
<i>glomerata</i> × <i>falcata</i> & reciprocal	22, 26
<i>glomerata</i> × <i>prostrata</i>	26
<i>glomerata</i> × <i>sativa</i>	26
<i>hybrida</i> × <i>suffruticosa</i>	27
<i>platycarpa</i> × <i>ruthenica</i>	52
<i>prostrata</i> × <i>falcata</i>	25
<i>prostrata</i> × <i>sativa</i> & reciprocal	25
<i>rhodopea</i> × <i>rupestris</i> & reciprocal	14, 30
F ₁ [<i>sativa</i> × <i>cancellata</i>] × <i>saxatilis</i>	28
<i>sativa</i> × <i>hybrida</i>	47
<i>sativa</i> × <i>marina</i>	47
<i>sativa</i> × <i>papillosa</i>	47
<i>sativa</i> × <i>rhodopea</i>	47
<i>sativa</i> × <i>rupestris</i>	47
F ₁ [<i>sativa</i> × <i>rhodopea</i>] × <i>saxatilis</i>	28
<i>sativa</i> × <i>saxatilis</i>	28
F ₁ [<i>sativa</i> × <i>saxatilis</i>] × F ₁ (<i>sativa</i> × <i>rhodopea</i>)	28
Perennial × Annual	
<i>sativa</i> × <i>scutellata</i>	59
Annuals	
<i>bonarotiana</i> × <i>rotata</i> & reciprocal = (× <i>blancheana</i>)	42
<i>ciliaris</i> × <i>intertexta</i>	43
<i>littoralis</i> × <i>tornata</i> & reciprocal	33, 64
<i>littoralis</i> × <i>truncatula</i> & reciprocal	17, 33, 63, 64
<i>muricoleptis</i> × <i>intertexta</i>	43
<i>muricoleptis</i> × <i>ciliaris</i>	43
<i>lesinsii</i> × <i>turbinata</i>	41
<i>laciniata</i> × <i>sauvagei</i>	65
<i>sauvagei</i> × <i>laciniata</i>	65
<i>soleirolii</i> × <i>tornata</i>	32
<i>truncatula</i> × <i>turbinata</i> & reciprocal	32

† Exchange hybridizations between subspecies of the *M. sativa* complex.

The color of its vegetative parts and its self-tripping mechanism were like those found in the annual parent.

3-7.2 Potential Uses of Wild *Medicago* Species as Alfalfa Genetic Resources

Alfalfa, the world's most important forage legume, is subject to varying degrees of loss from a large number of diseases and pest insects. In addition, expanding the cultivation of alfalfa to include less suitable areas would be desirable, and further improvements in quality would be beneficial. All of these problems can be solved best by plant breeding.

The use of wild (exotic, weedy, or unimproved) germplasm in incorporating useful agronomic traits into the cultivated germplasm has been demonstrated countless times. Thus, genes for the agronomic traits, including winter hardiness, creeping root habit, and resistance to some foliar diseases have been added to cultivated alfalfa from wild *M. sativa* ssp. *falcata* (2). The use of this subspecies, adapted to colder habitats, has been invaluable in extending alfalfa production to more northern regions.

Vasilchenko (77) described in detail the potential uses of various *Medicago* spp. in alfalfa improvement. Accessions of *M. sativa* ssp. \times *varia* from Tien Shan (People's Republic of China) may be resistant to environmental stress, because of their adaptation to a harsh environment and to rocky soils; while those found growing in Tibet are adapted to altitudes of 5000 m. Resistance to drought is present in accessions of *M. sativa* ssp. *falcata*, *M. sativa* ssp. *polychroa*, *M. dzhawaketica*, and *M. cancellata*. *Medicago marina* and *M. littoralis*, endemic to seashores, might be resistant to salinity. The former species is characterized by creeping, subterranean stems, suggesting its use in dune stabilization. The annual species, *M. truncatula*, *M. littoralis*, *M. rugosa*, *M. scutellata*, and *M. tornata* have good potential as annual forages. Some of these are cultivated for this purpose in Australia (79).

Most breeding efforts to date have concentrated on species within the *M. sativa* complex. The use of less related species in breeding work will require a substantial effort on the collection, preservation, description, and preliminary screening of germplasm. Some *Medicago* spp. have been screened, especially for disease and insect resistance. Renfro and Sprague (58) found that *M. dzhawaketica* and *M. suffruticosa* have a high level of resistance to spring blackstem (*Phoma medicaginis*), and that *M. arborea* had resistance to bacterial wilt (*Corynebacterium insidiosum*). Resistance to *Stemphylium* leafspot was reported in *M. cancellata* (7). Among the annual *Medicago* spp., *M. littoralis*, *M. murex*, *M. rigidula*, *M. tenoreana*, and *M. truncatula* exhibited a very high level of anthracnose resistance to both race 1 and 2; and a high level of resistance to both races of anthracnose was found in *M. arborea* (11). See Chapters 21 and 27 in this book for additional information on sources of disease resistance.

High levels of resistance to the potato leafhopper [*Empoasca fabae* (Harris)] and alfalfa weevil [*Hypera postica* (Gyllenhal)], major insect pests of alfalfa, has been shown in a few annual *Medicago* spp.; namely *M. disciformis*, *M. minima*, *M. polymorpha*, *M. rugosa*, *M. scutellata*, and *M. truncatula* (3,61,62). The mechanism for this insect resistance is the presence of gland tipped hairs (62). See Chapters 22 and 28 in this book for additional information on sources of insect resistance.

All of these sources of agronomic traits is worthless unless these traits can be transferred to cultivated alfalfa. A major problem in the utilization of the wild *Medicago* spp. is that little is known about their crossing

relationships to *M. sativa*. Several crossing barriers have been identified such as unequal ploidy levels (23,50), chromosome rearrangement (35), nonlethal chlorophyll deficiency (23,33,65), very slow pollen tube growth (60), and poorly developed seeds (25,33,59).

Methods for overcoming these barriers have involved the use of colchicine to double the chromosome number: (i) for hybridization to occur at equal ploidy levels (24,28,30); (ii) to accomplish hybridization at unequal ploidy levels (23,50); and (iii) to restore fertility in nonfertile hybrids (32). The discovery of a diploid male-sterile *M. sativa* (CADL) (49) has enhanced the interspecific hybridization efforts (46,49) (see Chapter 24 in this book). Also, phytohormones have been used to overcome crossing barriers. Sangduen et al. (59) applied gibberellic acid to the pedicels and peduncles immediately after pollination so that the hybrid pods were retained long enough for the development of mature seed. Researchers in other crops have used phytohormones to enhance pollen tube growth (9,12,21). The use of tissue culture and genetic engineering technology such as in vitro pollination, embryo rescue (81), protoplast fusion (85), and gene splicing (82, 83) will be useful in the near future for breaking crossing barriers and transferring agronomically useful traits. Recently, McCoy (48) reported the use of ovule-embryo culture techniques to rescue aborting interspecific hybrid embryos. See Chapter 24 in this book for further information on the use of these techniques.

No matter which method is used to unlock genetic barriers, the potential exists for the transfer of new and unique sources of pest resistance and for significant improvement in adaptation and yield characteristics.

3-8 SUMMARY

The genus *Medicago* comprise more than 60 different species among annuals and perennials. The primary center of diversity for the genus is found in the Caucasus, northwestern Iran and northeastern Turkey. The basic genomic number of *Medicago* is $x=8$, except for a few species which have a genomic number of $x=7$. Diploid, tetraploid, and hexaploids species occur in the genus.

The evolution of *Medicago* might have taken place in the Tertiary period. The annual species which are autogamous might have originated from allogamous perennial species. Taxonomically, the *Medicago* species are distinguished mainly by legume and seed characteristics, pubescence, pollen grain morphology, and chromosome numbers. Other biochemical traits might be used successfully for this purpose. The key to *Medicago* spp. of Lesins and Lesins (40), with a few modifications is included as a guide for species identification.

The *M. sativa* complex includes the taxa that hybridize freely with alfalfa. Therefore, they can be classified as subspecies of the species *M. sativa* on the basis of morphological and ploidy criteria. The *M. sativa* ssp. recognized in this chapter are: ssp. *sativa*, ssp. *coerulea*, ssp. *falcata*,

ssp. \times *varia*, ssp. \times *hemicycla*, ssp. \times *polychroa*, ssp. \times *tunetana*, and ssp. *glutinosa*. *Medicago glomerata* and *M. prostrata* are two other species closely related to the complex. The former species has been considered ancestral to *M. sativa* giving rise to the diploids *M. sativa* ssp. *coerulea* and *M. sativa* ssp. *falcata*. The tetraploid subspecies *M. sativa* ssp. *sativa* and *M. sativa* ssp. *falcata* likely originated from the diploids via unreduced gametes. On the basis of isozyme studies, it was found that among the diploid taxa, the most variable are the subspecies *M. sativa* ssp. \times *hemicycla* and *M. sativa* ssp. *falcata* as measured by numbers of alleles and percentage heterozygosity. In general, tetraploid subspecies were found to be more variable than diploids. Nevertheless, the percentage heterozygosity of the diploid *M. sativa* ssp. \times *hemicycla* was similar to that of the tetraploid *M. sativa* ssp. \times *varia* but higher than that found in cultivated alfalfa. Twice as many tri- and tetraallelic plants were found in natural tetraploids of *M. sativa* than in cultivated alfalfa. The Hairy Peruvian strain was the richest in tetraallelic plants among those tested. *Medicago sativa* ssp. *falcata* besides its characteristic yellow flowers and straight pods, has unique isozymes that can be used to determine if this subspecies is involved in the genetic composition of alfalfa cultivars that differ in origin.

An account of the interspecific hybridization in the genus is given in this chapter. The use of unimproved germplasm as an alfalfa genetic resource is documented. The potential of various wild species as sources of disease, insect, and stress resistance is mentioned. Possible strategies to achieve interspecific hybridization, especially for wide crosses are suggested.

ACKNOWLEDGMENT

The authors are indebted to the late Ernest H. Stanford and James H. Elgin, Jr. for reviewing the manuscript. Part of this work was supported by the Alberta Research Council grant DB102 to R.C. von Borstel and grant NSERC GR2 to the president of the Univ. of Alberta, Canada.

REFERENCES

1. Armstrong, J.M., and D.R. Gibson. 1941. Inheritance of certain characters in the hybrid of *Medicago media* and *M. glutinosa*. *Sci. Agric.* 22:1-10.
2. Barnes, D.K., E.T. Bingham, R.P. Murphy, O.J. Hunt, D.F. Beard, W.H. Skrdla, and L.R. Teuber. 1977. Alfalfa germplasm in the United States: Genetic vulnerability, use, improvement, and maintenance. USDA-ARS Tech. Bull. 1571. U.S. Government Printing Office, Washington, DC.
3. ———, and R.H. Ratcliffe. 1969. Evaluation of annual species of *Medicago* as sources of alfalfa weevil resistance. *Crop Sci.* 9:640-642.
4. Baughan, G.R., and J.H. Elgin, Jr. 1984. A new chromosome number for the genus *Medicago*. *Crop Sci.* 24:193-195.
5. Beckmann, J.S., and M. Soller. 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theor. Appl. Genet.* 67:35-43.

6. Bingham, E.T. 1980. Maximizing heterozygosity in autotetraploids. In W.H. Lewis (ed.) Polyploidy: Biological relevance. Plenum Publishing Corp., New York.
7. Borges, O.L., E.H. Stanford, and R.K. Webster. 1976. Sources and inheritance of resistance to *Stemphyllium* leafspot of alfalfa. *Crop Sci.* 16:458-461.
8. Clement, Jr., W.M. 1963. Chromosome relationships and diploid hybrid between *Medicago sativa* L. and *M. dzhawakhetica* Bordz. *Can. J. Genet. Cytol.* 5:427-435.
9. Crane, M.B., and E. Marks. 1952. Pear-apple hybrid. *Nature (London)* 170:1017.
10. Dubier, M.W., and E.T. Bingham. 1975. Maximum heterozygosity in alfalfa: results using haploid derived autotetraploids. *Crop Sci.* 15:527-531.
11. Elgin, Jr., J.H., and S.A. Ostazeski. 1982. Evaluation of selected alfalfa cultivars and related *Medicago* species for resistance to race 1 and race 2 anthracnose. *Crop Sci.* 22:39-42.
12. Emsweller, S.L., and N.W. Stuart. 1948. Use of growth regulation substances to overcome incompatibilities in *Lilium*. *Proc. Am. Soc. Hort. Sci.* 51:581-589.
13. Gillies, C.B. 1970. Alfalfa chromosomes. I. Pachytene karyotype of a diploid *Medicago falcata* L. and its relationship to *M. sativa* L. *Crop Sci.* 10:169-171.
14. ———. 1972. Pachytene chromosomes of perennial species. II. Species closely related to *M. sativa*. *Heredity* 72:277-288.
15. Gunn, C.R., W.H. Skrdla, and H.C. Spencer. 1978. Classification of *Medicago sativa* L. using legume characters and flower colors. USDA-ARS Tech. Bull. 1574. U.S. Government Printing Office, Washington, DC.
16. Harlan, J.R., and J.M.J. de Wet. 1975. On O. Winge and a prayer: The origins of polyploidy. *Bot. Rev.* 41:361-390.
17. Heyn, C.C. 1963. The annual species of *Medicago*. *Scr. Hierosolymitana* 12:1-154. Magnes Press, Jerusalem.
18. Ho, K.M., and K.J. Kasha. 1972. Chromosome homology at pachytene in diploid *Medicago sativa*, *M. falcata* and their hybrids. *Can. J. Genet. Cytol.* 14:829-838.
19. Ignasiak, T., and K. Lesins. 1975. Carotenoids in petals of perennial *Medicago* species. *Biochem. Syst. Ecol.* 2:177-180.
20. Ivanov, A.I. 1977. History, origin and evolution of the genus *Medicago*, subgenus *Falcago*. *Bull. Appl. Bot. Genet. Select.* 59:3-40 (Trudy po prikladnoy botanike, genetike i seleksii. Translation by the Multilingual Services Div. Dep. of the Secretary of State, Canada).
21. Larter, E., and C. Chaubey. 1965. Use of exogenous growth substances on promoting pollen tube growth and fertilization in barley-rye crosses. *Can. J. Genet. Cytol.* 7:511-518.
22. Lesins, K. 1952. Some data on the cytogenetics of alfalfa. *J. Hered.* 43:287-291.
23. ———. 1961a. Interspecific crosses involving alfalfa. I. *Medicago dzhawakhetica* (Bordz.) \times *M. sativa* L. and its peculiarities. *Can. J. Genet. Cytol.* 3:135-152.
24. ———. 1961b. Interspecific crosses involving alfalfa. II. *Medicago cancellata* M. B. \times *M. sativa* L. *Can. J. Genet. Cytol.* 3:316-324.
25. ———. 1962. Interspecific crosses involving alfalfa. III. *Medicago sativa* L. \times *M. prostrata* Jacq. *Can. J. Genet. Cytol.* 4:14-23.
26. ———. 1968. Interspecific crosses involving alfalfa. IV. *Medicago glomerata* \times *M. sativa* with reference to *M. prostrata*. *Can. J. Genet. Cytol.* 10:536-544.
27. ———. 1969. Relationship to taxa in genus *Medicago* as revealed by hybridization. IV. *M. hybrida* \times *M. suffruticosa*. *Can. J. Genet. Cytol.* 11:340-345.
28. ———. 1970. Interspecific crosses involving alfalfa. *Medicago saxatilis* \times *M. sativa* with reference to *M. cancellata* and *M. rhodopea*. *Can. J. Genet. Cytol.* 14:221-226.
29. ———. 1971. Interspecific crosses involving alfalfa. VI. Ineffectiveness of allopolyploidy in induction fertility in *Medicago pironae* \times *M. daghestanica* hybrids. *Can. J. Genet. Cytol.* 13:437-442.
30. ———. 1972. Interspecific crosses involving alfalfa. VII. *Medicago sativa* \times *M. rhodopea*. *Can. J. Genet. Cytol.* 14:221-226.
31. ———. 1970. Alfalfa, lucerne. *Medicago sativa* (Leguminosae-Papilionatae), p. 165-168. In N.W. Simmonds (ed.) Evolution of crop plants. Longman Group, London.
32. ———, J. Dickson, and L. Ostafichuk. 1980. Relationship of taxa in *Medicago* as revealed by hybridization. IX. *M. turbinata* \times *M. truncatula*. *Can. J. Genet. Cytol.* 22:137-142.
33. ———, and A. Erac. 1968. Relationship of taxa in the genus *Medicago* as revealed by hybridization. I. *M. striata* \times *M. littoralis*. *Can. J. Genet. Cytol.* 10:263-275.
34. ———, and C.B. Gillies. 1968. Relationship of taxa in the genus *Medicago* as revealed by hybridization. II. *M. pironae* \times *M. daghestanica* with reference to *M. sativa*. *Can. J. Genet. Cytol.* 10:454-459.
35. ———, and C.B. Gillies. 1972. Taxonomy and cytogenetics of *Medicago*. In C.H. Hanson (ed.) Alfalfa science and technology. *Agronomy* 15:53-86.
36. ———, and I. Lesins. 1960. Sibling species in *Medicago prostrata* Jacq. *Can. J. Genet. Cytol.* 2:416-417.

37. ———, and ———. 1963. Pollen morphology and species relationship in *Medicago* L. *Can. J. Genet. Cytol.* 5:270–280.
38. ———, and ———. 1964. Diploid *Medicago falcata* L. *Can. J. Genet. Cytol.* 6:152–163.
39. ———, and ———. 1966. Little known *Medicagos* and their chromosome complements. IV. Some mountain species. *Can. J. Genet. Cytol.* 8:8–13.
40. ———, and ———. 1979. Genus *Medicago* (*Leguminosae*). A taxogenetic study. Junk, The Hague, Netherlands.
41. ———, ———, and C.B. Gillies. 1970. *Medicago murex* with $2n=16$ and $2n=14$ chromosome complements. *Chromosoma* 30:109–122.
42. ———, R.S. Sadasivaiah, and S.M. Singh. 1976. Relationship of taxa in the genus *Medicago* as revealed by hybridization. VIII. Section *Rotatae*. *Can. J. Genet. Cytol.* 18:345–355.
43. ———, S.M. Singh, and A. Erac. 1971. Relationship of taxa in the genus *Medicago* as revealed by hybridization. V. Section *Intertextae*. *Can. J. Genet. Cytol.* 13:335–346.
44. Lubenets, P.A. 1972. Alfalfa-*Medicago* L. A brief survey of the genus and the classification of the subgenus *Falcago* (Rehb.) Grossh. *Bull. Appl. Bot. Genet. Select. Forage Crops* 47:3–68. (Trudy po prikladnoi botanike, genetike i selektsii; kormovye kul'tury. Translation by the Multilingual Service Div., Dep. of the Secretary of State, Canada).
45. Mariani, A., and F. Veronesi. 1979. Cytological and fertility relationships of different *Medicago* species and cytogenetic behavior of their hybrids. *Genet. Agric.* 33:245–268.
46. McCoy, T.J. 1982. The inheritance of $2n$ pollen formation in diploid alfalfa. *Medicago sativa*. *Can. J. Genet. Cytol.* 24:315–323.
47. ———. 1984. Interspecific hybrids of perennial *Medicago* species produced via ovule-embryo culture. p. 56. In D.R. Viands (ed.) 29th National Alfalfa Improvement Conf., Lethbridge, AB, Canada. 15–20 July. National Alfalfa Improvement Conf., Lethbridge, AB, Canada.
48. ———. 1985. Interspecific hybridization of *Medicago sativa* and *M. rupestris* MB. using ovule-embryo culture. *Can. J. Genet. Cytol.* 27:238–245.
49. ———, and L.Y. Smith. 1983. Genetics, cytology, and crossing behavior of an alfalfa (*Medicago sativa*) mutant resulting in failure of the postmeiotic cytokinesis. *Can. J. Genet. Cytol.* 25:390–397.
50. ———, and ———. 1984. Uneven ploidy levels and a reproductive mutant required for interspecific hybridization of *Medicago sativa* and *M. dzhawakhetica* Bordz. *Can. J. Genet. Cytol.* 26:511–518.
51. McLennan, H.A., J.M. Armstrong, and K.J. Kasha. 1966. Cytogenetic behavior of alfalfa hybrids from tetraploid by diploid crosses. *Can. J. Genet. Cytol.* 8:544–555.
52. Oldemeyer, R.K. 1956. Interspecific hybridization in *Medicago*. *Agron. J.* 48:584–585.
53. Pfeiffer, T.W., and E.T. Bingham. 1983. Abnormal meiosis in alfalfa, *Medicago sativa*: Cytology of $2n$ egg and $4n$ pollen formation. *Can. J. Genet. Cytol.* 25:107–112.
54. Quiros, C.F. 1982. Tetrasomic segregation for multiple alleles in alfalfa. *Genetics* 101:117–127.
55. ———. 1983. Alfalfa, luzerne (*Medicago sativa* L.) p. 253–294. In S.D. Tanksley and T.J. Orton (ed.) *Isozymes in plant genetics and breeding, Part B*. Elsevier Scientific Publishing Co., Amsterdam.
56. ———, and K. Morgan. 1981. Peroxidase and leucine-aminopeptidase in diploid *Medicago* species closely related to alfalfa: Multiple gene loci, multiple allelism and linkage. *Theor. Appl. Genet.* 50:221–228.
57. ———, and L. Ostafichuk. 1983. Allozymes and genetic variability in *Medicago turbinata*, *M. truncatula*, and their hybrid. *Can. J. Genet. Cytol.* 25:286–291.
58. Renfro, B.L., and E.W. Sprague. 1959. Reaction of *Medicago* species to eight alfalfa pathogens. *Agron. J.* 51:481–483.
59. Sangduen, N., E.L. Sorensen, and G.H. Liang. 1982. A perennial \times annual *Medicago* cross. *Can. J. Genet. Cytol.* 24:361–365.
60. ———, ———, and ———. 1983. Pollen germination and pollen tube growth following self-pollination and intra- and interspecific pollination of *Medicago* species. *Euphytica* 32:527–534.
61. Shade, R.E., M.J. Daskocil, and N.O. Maxon. 1979. Potato leafhopper resistance in glandular-haired alfalfa species. *Crop Sci.* 19:287–288.
62. ———, T.E. Thompson, and W.R. Campbell. 1975. An alfalfa weevil larval resistance mechanism detected in *Medicago*. *J. Econ. Entomol.* 8:399–494.
63. Simon, J.P. 1965. Relationship in annual species of *Medicago*. II. Interspecific crosses between *M. tornata* (L.) Mill. and *M. littoralis* Rhode. *Austr. J. Agric. Res.* 16:51–60.
64. ———, and A.J. Millington. 1967. Relationship in annual species of *Medicago*. III. The complex *M. littoralis*-*M. truncatula* Gaertn. *Aust. J. Bot.* 15:35–73.
65. Singh, S.M., and K. Lesins. 1972. Relationship of taxa in the genus *Medicago* as revealed by hybridization. VI. *M. laciniata* \times *M. savagei*. *Can. J. Genet. Cytol.* 14:823–828.
66. Sinskaya, E.N. 1961. Flora of cultivated plants of the U.S.S.R. XIII. Perennial leg-

- uminous plants. Israel Program for Scientific Translations, Jerusalem, National Science Foundation Pub., Washington, DC.
57. Small, E. 1981. A numerical analysis of major groupings in *Medicago* employing traditionally used characters. *Can. J. Bot.* 59:1553-1577.
 58. ———, I.J. Bassett, and C.W. Crompton. 1981. Pollen variation in tribe *Trigonella* (Leguminosae) with special reference to *Medicago*. *Pollen Spores* 23:295-320.
 59. ———, and G.R. Bauchan. 1984. Chromosome numbers of the *Medicago sativa* complex in Turkey. *Can. J. Bot.* 62:749-752.
 70. ———, and B.S. Brookes. 1984a. Taxonomic circumscription and identification in the *Medicago sativa-falcata* (alfalfa) continuum. *Econ. Bot.* 38:83-96.
 71. ———, and ———. 1984b. Reduction of the geocarpic *Factorovskya* to *Medicago*. *Taxon* 33:622-635.
 72. ———, and ———. 1985. *Medicago lesinsii*, a new Mediterranean species. *Can. J. Bot.* 63:728-734.
 73. ———, and P. Lefkovitch. 1982. Agrochemotaxometry of alfalfa. *Can. J. Plant Sci.* 62:919-928.
 74. Smith, S.E., R.P. Murphy, and D.R. Viands. 1984. Reproductive characteristics of hexaploid alfalfa derived from 3x-6x crosses. *Crop Sci.* 24:169-172.
 75. Sprague, E.W. 1959. Cytology and fertility relationships of *Medicago sativa*, *M. falcata*, and *M. gaetula*. *Agron. J.* 51:249-252.
 76. Stanford, E.H. 1951. Tetrasomic inheritance in alfalfa. *Agron. J.* 43:222-225.
 77. Vasilchenko, I.T. 1949. Flora and systematics of higher plants: Alfalfa—the best forage plant. *Trans. V. L. Komarov Bot. Inst. Acad. Sci. USSR* 8:9-421. (Trudy Botanicheskogo Instituta Imeni V. C. Komarova Akademii Nauk Soyuz Sotsialisticheskikh Respublik. Translation by the Multilingual Service Div. Dep. of the Secretary of State, Canada).
 78. Vorsa, N., and E.T. Bingham. 1979. Cytology of 2n pollen formation in diploid alfalfa, *Medicago sativa*. *Can. J. Genet. Cytol.* 21:525-530.
 79. von Borstel, R.C., and K. Lesins. 1977. On germplasm conservations with special reference to the genus *Medicago*. In A. Muhammed, et al. (ed.) *Genetic diversity in plants*. Basic Life Sciences, Vol. 8. Plenum Press, New York.
 80. Yen, S.T., and R.P. Murphy. 1979. Cytology and breeding of hexoploid alfalfa. I. Stability of chromosome number. *Crop Sci.* 19:389-393.

ADDITIONAL REFERENCES

81. Bauchan, G.R. 1987. Embryo culture of *Medicago scutellata* and *M. sativa*. *Plant Cell Tissue Organ Cult.* 10:21-29.
82. Deak, M., G.B. Kiss, C. Konecz, and D. Dudits. 1986. Transformation of *Medicago* by *Agrobacterium* mediated gene transfer. *Plant Cell Rep.* 5:97-100.
83. Shahin, E.A., A. Spielmann, K. Sukhapinda, R.B. Simpson, and M. Yashar. 1986. Transformation of cultivated alfalfa using disarmed *Agrobacterium tumefaciens*. *Crop Sci.* 26:1235-1239.
84. Small, E. 1984. A clarification of the *Medicago blanchiana-bonariotiana-rotate* complex. *Can. J. Bot.* 62:1693-1696.
85. Téoulé, E. 1983. Hybridation somatique entre *Medicago sativa* L. et *M. falcata* L. *C.R. Acad. Sci. Ser. 3*, 297:13-16.