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LIMITATIONS TO WIDE CROSSES IN CLOVER AND LUCERNE BREEDING

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Among the limitations to wide crosses between species of forage legumes are divergent chromosome numbers and ploidy levels or generally the lack of genetic affinity. Limitations can be reduced by either raising or decreasing ploidy levels, temperature treatment and embryo and tissue culture techniques. Chemical analysis revealed groups of species with different genetic affinity. No interspecific crosses between cultivated *Trifolium* species have been obtained. Artificial hybrids involving cultivated and wild *Trifolium* species were of little agronomic value. The development of low coumarin sweet clover by interspecific hybridization of *Melilotus alba* and *M. dentata* is of practical importance. A considerable number of species crosses has been produced in the genus *Medicago* at both the diploid and tetraploid level involving *M. sativa, M. varia, M. falcata* und other perennial species. Crosses between annual and perennial species were unsuccessful.

INTRODUCTION

Clover and lucerne species are of worldwide importance as sources of forage. Often the cultivated forms do not differ significantly from their wild ancestors. The need to introduce valuable germplasm from primitive forms into cultivated forage species appears to be less urgent as compared with certain other field crops. A tremendous number of species is available as actual or potential sources for breeding purposes. The original habitat as well as the region of early cultivation of the traditional forage species encompass the temperate and mediterranean climatic zones of Europe and the Near and Middle East. Interest in tropical and subtropical clovers and cloverlike species started only recently. Their number of species and production potential appears to be at least as large as that of the traditional leguminous forage species. A selection of forage legume genera adapted to different climatic zones is presented in Table 1.

Table 1. – Forage legumes genera

Temperate and mediterranean
Coronilla
Hedysarum
Lotus
Lupinus
Medicago
Melilotus
Onobrychis
Ornithopus
Trigonella
Trifolium
Vicia

Subtropical and Tropical
Aeschynomene
Alysicarpus
Arachis
Astragalus
Cajanus
Calopogonium
Cassia
Centrosema
Desmodium
Glycine
Indigofera
Lathyrus
Lespedeza
Leucaena
Macroptilium
Mimosa
Pachyrrhizus
Pueraria
Stylosanthes
Teramnus
Vigna
Zornia

The wording „wide crosses" is rather difficult to define as far as forage legumes are concerned. Some authors confine the term to crosses between species. The trouble is that in several important genera the limits between species are not well established. On the other hand, there are very variable species where the mating of extremely different genotypes may well be considered a „wide cross". Definitions notwithstanding, the reasons for making wide crosses are essentially the same is in other cultivated plants: namely, to widen the genetic basis of the breeding stock and, by introducing valuable germplasm, to improve yield, adaptation to environment and management, resistance to pathogens, and forage quality.

In practice the results of wide crosses are often rather disappointing. This is particularly so if parents differ in chromosome number or ploidy level. Even if they do not, the genetic affinity between genotypes may be too small to make hybridization successful.

Various procedures have been adapted to overcome the limitations to wide crosses. Chromosome levels of crossing partners can be raised by colchicine or lowered by haploidization (Bingham, 1971). Temperature treatment may be effectively used in obtaining interspecific hybrids involving red clover (Anderson and Taylor, 1974). Smith (1964) used a grafting technique in producing interspecific hybrids in sweetclover. Also new methods of plant genetic manipulation like embryo and tissue culture have been successfully applied in several clover species. Since the demonstration of induced inter–species fusion of isolated protoplasts, there has been an effort to utilize the resulting heterokaryons in a breeding programme. The most realistic application of protoplast fusions will probably involve intergeneric somatic hybridizations, for example between closely related genera, such as *Medicago* and *Trifolium*. An account of various traditional and new methods of genetic manipulation in exploring extra–specific genetic variation in legumes is given by Razdan and Cocking (1981).

In order to predict successful interspecific hybridization, several workers (Anonymus, 1972; Abdel–Tawab *et al.*, 1976; Selim *et al.*, 1976; Quiros, 1981) use chromatographic methods for the establishment of groups of species with close genetic affinity.

WIDE CROSSES IN SELECTED GENERA AND SPECIES

A plant species named clover does not necessarily belong to the botanical genus *Trifolium*. There are many species of other genera which are termed clover in this or another language. Such genera include, for example *Hedysarum, Lotus, Medicago, Melilotus, Onobrychis, Trigonella*. However, the genus *Trifolium* contains the largest number of cultivated species, and these species surpass clovers of other genera except *Medicago* in world–wide importance.

Table 2. – Clover genera and species

Trifolium	Chromosomes (n)	*Trifolium*	Chromosomes (n)
Cultivated			
alexandrinum	8	repens	16
hybridum	8	resupinatum	8
incarnatum	7	subterraneum	8,6
pratense	7		
Wild			
alpestre	8	lappaceum	8
ambiguum	8	medium	aneuploid
canescens	9	nigrescens	8
cherleri	5	noricum	8
diffusum	8	pallidum	8
fragiferum	8	rubens	8
heldreichianum	8	sarosiense	24
hirtum	5	striatum	7

Trifolium. – In table 2 the economically important *Trifolium* species are listed. Some indigenous species are added that were used for hybridization with cultivated clovers. Up to now, a large number of interspecific crosses has been made. The results, however, are disappointing. Of the 47 artificial interspecific crosses reported by Ullmann (1938), only one, *T. pratense* x *T. medium*, resulted in viable progeny. A successful cross between *T. pratense* and *T. repens* would be of particular interest because another species of economic importance, *T. hybridum*, was thought by Linne to be a hybrid between those species. Although earlier *T. pratense* x *T. repens* crosses failed, Starzycki (1969, 1972) claimed to have succeeded in producing viable plants of such hybrids after irradiation of the mother plants with a gamma–ray dose of 6450 R 15 days before pollination.

Of 11 *Trifolium* species used in hybridization by Selim et al. (1977), viable hybrid seed was obtained only from *T. repens* x *nigrescens*, *T. nigrescens* x *hybridum*, and *T. pratense* x *medium*. According to Taylor & Smith (1979) the only verified hybrids involving red clover were obtained with the diploid annuals *T. diffusum* and *T. pallidum*. Both interspecific hybrids were of no value as sources of genetic variation. Apparently, the wild species involved in the crosses do not possess sufficiently different genes to be useful as a source of genetic material for the improvement of *T. pratense*. The interspecific hybrids of perennial *Trifolium* species reported by Taylor & Smith (1979) are of little direct interest to the breeder of cultivated clover species according to these authors.

No hybrids were produced between *T. pratense* and perennial species except *T. medium* and the closely related species *T. sarosiense*. Phillips *et al.* (1982) succeeded in the hybridization of *T. pratense* and *T. sarosiense* using an *in vitro* embryo rescue technique. Immature hybrid embryos were excised and cultured for 8 to 14 days on a certain medium. Viable embryos were then transferred to a standard medium. About 10 % of the hybrid embryos were rescued using the optimal culture sequence. Some embryos produced only callus from which plants were regenerated using an alternate culture scheme. Similarly a hybrid between Caucasian clover, *T. ambiguum*, and white clover, *T. repens*, has been produced by Williams (1978) with the aid of embryo culture using transplanted nurse endosperm. *T. ambiguum* is a cold–hardy perennial, resistant to drought and several viruses which attack the fast growing and commonly used *T. repens*.

The primary cause of the failure of *T. pratense* crosses with related perennials appear to be post–zygotic mechanisms (Phillips *et al.*, 1982). Principal among these mechanism is endosperm failure followed by embryo abortion.

No problem is presented in the hybridization of cultivated red clover with its wild relative, *T. pratense* ssp. *spontaneum*, which is common in perennial grassland throughout Europe. It is believed (Julen, 1959) to possess valuable genes for winter hardiness and persistence, but its yield potential is much lower than that of cultivated red clover (Korosec *et al.*, 1980; Goral, 1982). The practical results of such crosses have so far been disappointing. There may be one exception, however. If one shares the opinion that the Swiss „Mattenklee" is a form of ssp. *spontaneum*, crosses between it and other cultivated red clover have led to valuable results. For example, three cultivars, 'Glauca', 'Lucrum' and 'Lutea', expressing superior winter hardiness, persistance and resistance to clover crown and root rot, were produced at the Bavarian State Plant Breeding Station at Weihenstephan from crosses between Mattenklee and Danish Lyngby red clover. Intraspecific crosses of agronomic value have also been made in *T. alexandrinum*, *T. repens*, *T. resupinatum*, and *T. subterraneum*.

For predicting successful interspecific hybridization within the genus *Trifolium,* authors have grouped species on the basis of chromatographic affinities. The probability of success is thought to be greater in crosses of species within groups rather than between groups. Thus, Anonymus (1972) found a close relationship of *Trifolium pratense* and *T. cherleri, lappaceum, diffusum,* and *pallidum.* Abdel – Tawab *et al.* (1976) established the following 4 groups of chromatographic affinities:

(a) *T. alexandrinum, pratense, lappaceum, hirtum, nigrescens*
(b) *T. alexandrinum, lappaceum, resupinatum, subterraneum*
(c) *T. pratense, medium, repens, hybridum, nigrescens*
(d) *T. fragiferum.*

Melilotus. – Sweet clover, *Melilotus alba* and *M. officinalis,* is extensively grown in the United States and Canada for forage production, but up to now has attracted little attention in Europe. Although rich in protein and minerals, its main disadvantage is the low palatability due to the content of coumarin. The production of low coumarin sweet clover is one of the major achievements of interspecific hybridization.

Among the many *Melilotus* species *M. dentata* is know to contain no coumarin. Several earlier attempts to transfer coumarin–free germplasm from *M. dentata* to either *M. alba* or *M. officinalis* failed. Smith (1964) succeeded in obtaining F_1 seed from *M. alba* x *dentata,* but the F_1 plants were chlorophyll deficient and could be reared to maturity only by grafting on normal green sweet clover plants. The F_1 hybrids set no seed on selfing, but a few seeds were obtained when flowers of the grafted hybrids were fertilized with pollen from *M. alba.* Vigour and productivity were combined with the low coumarin character by repeating four times the programme of backcrossing.

Medicago. – Taxonomy. Lucerne is certainly the oldest cultivated forage species and probably the most widely grown forage crop throughout the world. Taxonomically, lucerne belongs to the genus *Medicago* L. It comprises more than 60 different species including annuals and perennials, diploids, tetraploids and hexaploids, with chromosome numbers that are multiples of $n = 7$ and $n = 8$. The annuals are predominantly autogamous species while the perennials are allogamous. Autogamous species do not intercross with allogamous (Quiros, 1979). For that reason, artificial crosses between annuals and perennials have been unsuccessful.

The basic taxa of cultivated perennial lucerne are *M. sativa, M. falcata,* and *M. glutinosa* (Stanford *et al.,* 1972). As they interbreed freely and the offspring have unimpaired viability, hybrid swarms are produced covering the range of variability between the parental species and have been given species designation. *M. falcata* and *M. sativa* are known to occur both at the diploid and tetraploid levels. There seems to be no barrier to the free interchange of genes between members of the complex that are at the same ploidy level. Also, if the diploid forms are raised to the tetraploid level, they hybridize readily with the tetraploid species and give rise to fertile hybrids.

As a result, a most confusing situation has arisen with regard to the designation and delimitation of the basic taxa of cultivated lucerne. Consequently, if we confine the term „wide crosses" to the hybridization between species or genera, it may or may not apply to a certain cross, depending on the taxonomic ranking of the parental genotypes. Originally, the basic types of cultivated lucerne, *M. sativa* and *M. falcata,* had been given species rank. Nowadays, however, the justification for this ranking order is being increasingly questioned. Lesins and Gillies (1972) and Stanford *et al.* (1972) avoid the species term for *M. sativa, M. falcata* and the related *M. glutinosa,* and the hybrid population arising from intercrossing these types which has been referred to as *M. media (varia).* The authors consider them as components of the *M. sativa–falcata–glutinosa* complex. Ehrendorfer (1973) puts them together in the aggregate *M. sativa* sensu lato.

Evolution of cultivated lucerne. – The hypothetical evolution of cultivated lucerne is summarized in figure 1 (Quiros, 1979). *Medicago glomerata,* a diploid species with yellow flowers and coiled pods, which is found in Northern Italy, gave rise to diploid *M. sativa* and *M. coerulea,* respectively, and diploid *M. falcata. M. coerulea* is characterized by purple flowers and coiled pods, and it is found below 42° latitude North. Diploid *M. falcata,* characterized by yellow flowers and cickleshaped pods, is found mostly at higher latitudes between 42 to 62° North in Europe and Western Asia. *M. hemicycla,* another diploid and presumably a natural hybrid between diploid *M. sativa (M. coerulea)* and diploid *M. falcata,* shows the whole range of variation between both putative parental species for flower colour and pod shape.

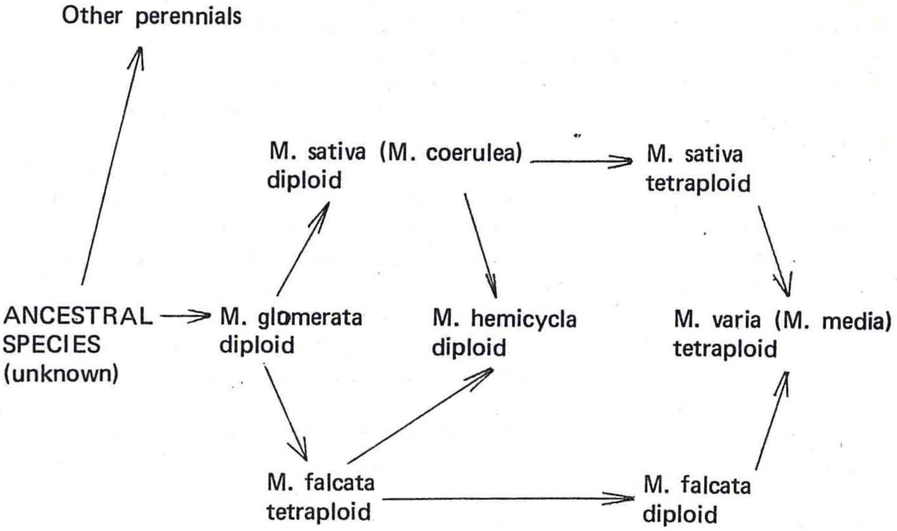

Figure 1. – Hypothetical evolution of cultivated lucerne (Quiros, 1979)

The tetraploid *M. sativa* and *M. falcata* originated from the diploids by natural doubling of the chromosome number by means of unreduced diploid gametes. *M. varia* (= *M. media*) originated by natural hybridization between both tetraploid species. Like the hybrid at the diploid level, *M. hemicycla,* it expresses the whole range of variation for flower colour and pod shape of both parental species. It is from the last three species that cultivated lucerne has been derived. Cultivars with mostly *M. sativa* background are adapted to southern latitudes, while those whith *M. falcata* germplasm are adapted to northern latitudes. Generally speaking, *M. sativa* is more productive than *M. falcata* under favourable growing conditions whereas *M. falcata* shows more resistance to an unfavourable environment. The breeder must decide which type of germplasm should be introduced into the breeding stock.

Crosses between ploidy levels. – Up to now, germplasm for the improvement of cultivated lucerne has been confined to the *M. sativa–falcata* complex although a great number of crosses between species have been made for various reasons. Crosses between diploids (2n = 16) and tetraploids (2n = 32) have been the most important interploid crosses in terms of transferring desirable germplasm for use in breeding.

According to Stanford *et al.* (1972) 5 to 15 progeny are produced per 1000 diploid–tetraploid or reciprocal crosses. Often the progeny are all tetraploid if the diploid is used as the female parent, and either triploid or tetraploid if the diploid is used as the male parent. Most of the embryos are triploid in either cross, but the majority abort within 6 days after fertilization, probably due to poor endosperm development. However, more triploids survive when the female has the higher chromosome number. Tetraploids are produced in either cross when gametes with the unreduced chromosome number (2n gametes) from the diploid parent unite with the reduced gametes from the tetraploid parent. Thus, polyploidy does not form a perfect isolation barrier between diploid and tetraploid forms.

Hybridation at the same ploidy level, however, is much more successful. Therefore, if the parental genotypes have different chromosome numbers, say 2n = 16 and 2n = 32, the success of hybridization can be increased by either raising the ploidy level of the diploid parent to that of the tetraploid one, or scaling down the tetraploid to the diploid level. The first method was applied by Lavrenteev and Chernenko (1976). They doubled by colchicine treatment the chromosome set of diploid *M. coerulea* and *M. erecta* before they were crossed with tetraploid *M. sativa.* Lesins, (1972) crossed diploid *M. sativa* with tetraploid *M. rhodopea* and obtained a triploid hybrid which was made hexaploid by colchicine treatment and then successfully crossed to hexaploid *M. sativa.*

The second method is the development of haploids (2n = 16) from tetraploid lucerne which was first demonstrated by Bingham (1971). Such haploids permit genetic and cytological research to be effected at the diploid level. In controlled tetraploid x diploid crosses, 1 to 10 haploids per 1000 pollinations are generally produced, presumably when gametes from the diploid fertilize only the endosperm mother cell and the egg develops parthenogenetically. Haploids and their diploid derivatives tend to produce more 2n gametes than wild diploids produce. Thus, they are comparatively crossable with tetraploids as well as diploids and even produce a few tetraploids in diploid x diploid matings (Stanford *et al.* (1972). Bingham and Saunders (1974) showed how cultivated tetraploid lucerne can be scaled to seven ploidy levels ranging from diploid to octoploid. Tetraploids were scaled down by haploidy. Scaling upwards was accomplished by sexual polyploidization, colchicine chromosome doubling, and spontaneous doubling in tissue cultures.

Successful "wide" crosses. – An incomplete list of successful „wide" crosses in the genus *Medicago* L. derived from various sources (Ullmann, 1938; Rudorf, 1959; Lesins and Gillies, 1972; Lavrenteev and Chernenko, 1976) is presented in Table 3. The relationship of other taxa to the *Medicago sativa* complex, as revealed by hybridization, is as follows in decreasing closeness of relationship: *M. glomerata, prostrata, saxatilis, cancellata, papillosa, rhodopea, daghestanica–pironae.* All of these are perennials. The other perennial species seem to be unrelated to the above taxonomic group as no hybrids have been obtained (Lesins and Gillies, 1972). Numerous crossing attempts involving other *Medicago* species and even species of related genera (*Melilotus, Trigonella*) remained without viable progeny.

Table 3. – Successful wide crosses in the genus Medicago L.
(Rudorf 1959; Lesins and Gillies, 1972;
Lavrenteev *et al.*, 1976).

(a) Between species of cultivated lucerne
M.sativa (32) x media (32), falcata (16,32)
M.media (32) x sativa (32), falcata (16,32)
M.falcata (16,32) x sativa (32), media (32)
M.glutinosa (32) x sativa (32)

(b) Between species of cultivated lucerne and wild perennial species
M.sativa (32) x coerulea (16), gaetula (32),
M. sativa (32) x polymorpha (14), prostrata (16,32)
M. sativa (32) x rhodopea (16), saxatilis (48)
M.falcata (16,32) x gaetula (32), prostrata (16,32)

(c) Between wild perennial species
M.deghestanica (16) x pironae (16)
M.platycarpa (16) x ruthenica (16)
M.rhodopea (16) x rupestris (16)

(d) Between wild annual species
M.blancheana (16) x rotata (16)
M.ciliaris (16) x intertexta (16)
M.intertexta (16) x muricoleptis (16)
M.laciniata (16) x sauvagei (16)
M.murex (16,14) x turbinata (16)

The question may be raised: „To what extent have wide crosses contributed to the creation of the present wealth of lucerne cultivars? " The answer, again, depends on whether or not the taxa involved in the *M. sativa–falcata* complex should be considered valid species. The question can even be narrowed to *M. sativa* alone because it is known to be extremely variable. Rudorf (1959) lists 16 distinct ecotypes, and the hybridization of morphologically or physiologically very different types may well be considered a wide cross. Barnes *et al.* (1977) estimated the genetic background of nearly 150 North American alfalfa cultivars. They found that most of them could be traced back to seven introductions of *M.*

sativa and to *M. falcata* and *M. varia* as well. More than half of the cultivars listed contain a proportion of *M. falcata* and *M. varia* germplasm ranging from 1 to 100%. Bingefors and Quittenbaum (1972) reported that the Swedish variety U 0635 derived from the cross (tetraploid *M. coerulea* x *M. sativa)* x Du Puits exceeded other Swedish cultivars in agronomic performance. Mariani *et al.* (1972) backcrossed *M. sativa* x *M. glutinosa* and *M. sativa* x *M. gaetula* hybrids and found the *M. sativa* x *M. glutinosa* backcrosses particularly well performing. In another paper, however, Mariani *et al.* (1976) state that *M. sativa* and *M. glutinosa* should not be considered as distinct species.

Importance of annual medics. – Until recently interest in lucerne as a source of forage has been confined to the *M. sativa–falcata* complex. Intensified research revealed that among the annual *Medicago* species some could serve as valuable sources of fodder particularly in arid zones. Among them are *M. rugosa*, *M. scutellata*, and *M. truncatula* which are already extensively grown in Australia. Several varieties of these species are listed in the OECD–List of Cultivars Eligible for Certification 1982. In a large screening programme of annual medics conducted at ICARDA, Somaroo and Witcombe (1982) found *M. rigidula*, which predominantly originated from Turkey to be particularly well adapted to higher elevation arid zones of Middle East and South West Asia. Interspecific hybridization may become equally important in the improvement of agronomic characters of annual medics as in the *M. sativa-falcata* complex.

Table 4. – Tropical legumes morphological diversity

(Kretschmer and Snyder, 1979).

Genus	MD	Species	MD
Aeschynomene	VL	americana	S
Calopogonium	S	mucunoides	S
Centrosema	L	pubescens	M
		virginianum	M
Desmodium	VL	heterocarpon	M
		intortum	S
		uncinatum	S
Glycine	M	wightii	S
Leucaena	L	leucocephala	S
Lotononis	L	bainesii	S
Macroptilium	L	atropurpureum	S
		bracteatum	S
Pueraria	S	phaseoloides	S
Stylosanthes	VL	guianensis	L
		hamata	M
		humilis	M
Teramnus	M	labialis	S
		uncinatus	S
		volubilis	S

MD = Morphological diversity: S = small; M = moderate; L = large; VL = very large

Tropical forage legumes. – Forage legumes that are productive in temperature zones are not necessarily useful in the tropics. Therefore, the existing wealth of tropical species is now being evaluated for its agronomic significance. Logically, emphasis is given at this initial stage of evaluation to the selection of adapted and productive plant types from natural populations rather than to the creation of wide crosses. Nevertheless, morphological diversity of a number of genera and species as shown in Table 4 (Kretschmer and Snyder, 1979) appears to be large enough to encourage breeders to utilize hybridization for the improvement of tropical legumes. Among the results of interspecific crosses of possible agronomic importance are *Leucaena leucocephala* x *L. pulverulata* and *Centrosema pubescens* x *C. macrocarpum* both of which are expected to be better adapted to acid soil low of fertility (Gwildis, 1982).

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OGRANIČENJA ZA UDALJENA UKRŠTANJA PRI OPLEMENJIVANJU DETELINE I LUCERKE

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Izvod

Među ograničenjima za udaljena ukrštanja između vrsta krmnih leguminoza su različit broj hromozoma i različiti nivoi ploidije, ili uopšteno govoreći nedostatak genetičkog afiniteta. Ograničenja se mogu smanjiti povećanjem ili smanjivanjem nivoa ploidije, temperaturnim tretmanom i korišćenjem tehnika kulture embria i kulture tkiva. Hemijskim analizama se utvrđuju grupe vrsta sa različitim genetičkim afinitetom. Do sada nisu dobijeni međuvrsni hibridi između gajenih vrsta *Trifolium*. Hibridi dobijeni ukrštanjem gajenih i divljih vrsta *Trifolium* imaju malu agronomsku vrednost. Dobijanje slatke deteline sa niskim sadržajem kumarina međuvrsnom hibridizacijom *Melilotus alba* i *M. dentata* ima i praktičnu vrednost. U rodu *Medicago* dobijen je veći broj specijes hibrida na diploidnom i tetraploidnom nivou, uključujući *M. sativa, M. varia, M. falcata* i druge višegodišnje vrste. Do sada nisu uspela ukrštanja između jednogodišnjih i višegodišnjih vrsta.

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