Clovers Around the World: A Symposium in Memory and Honor of Dr. Norman L. Taylor

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Introduction

Trifolium is arguably the largest and most diverse genera of herbaceous forage legumes (Zohary and Heller, 1984). Dr. Norman L. Taylor, Professor Emeritus, Department of Plant and Soil Science, University of Kentucky, deceased, was a pioneer and champion of collection and preservation of *Trifolium* species throughout his 57-year career. Dr. Taylor was a native of northern Kentucky, born near where this Conference is being held.

Norman L. Taylor

Dr. Taylor obtained his B.S. and M.S. from the University of Kentucky (UK) and then a Ph.D. from Cornell University in 1953. He returned to an academic position at UK in 1953 and progressed through the academic ranks from Assistant Agronomist to Professor, a position he held from 1965 to 2001. Following his "retirement" he dedicated an additional nine years of service as Emeritus Professor until his passing in 2010.

Dr. Taylor had a distinguished career as a forage legume breeder [primarily red clover (*T. pratense* L.)] with pioneering publications on interspecific hybridization among *Trifolium* species (Taylor, et al., 1963; Taylor and Gillett, 1988; Taylor, et al., 1994; Quesenberry and Taylor, 1976, 1977, 1978), clover breeding methodology (Taylor and Smith, 1979), ploidy manipulation (Taylor, et al., 1976), and genetic systems such as double cross hybrids (Anderson, et al., 1972), genetic system relationships (Taylor, et al., 1979), a crop science breeding summary paper (Taylor, 2008), and clover overview books (Taylor, 1985; Taylor and Quesenberry, 1996). He released signal cultivars of red clover ['Kenstar' (Taylor and Anderson, 1973) and 'Freedom MR' (Taylor, 2008)], kura clover ['Rhizo' (Henry and Taylor, 1989)], and crimson clover ('Kentucky Pride'). He taught a diverse group of classes in crop science, was instrumental in developing and coordinating the Crop Science Ph.D. graduate program, served as interim department chair, and on many university committee assignments. He was a mentor and advisor to a host of graduate students, including the senior author of this paper.

Dr. Taylor was perhaps best known worldwide for his efforts in collecting, maintaining, evaluating, and distributing the largest collection of *Trifolium* species assembled at one location. He participated in five United States Department of Agriculture (USDA) Agriculture Research Service (ARS) National Plant Germplasm System, Plant Exchange Office (PEO) organized and funded germplasm collection expeditions (Greece, Crete and Italy - 1977; Romania - 1984; Yugoslavia - 1988; California – 1994; Oregon and Washington – 1994; and Baja Mexico 2000) and actively evaluated accessions from these collections. Norman spent a sabbatical in New Zealand where he worked with Dr. Margot Forde to grow out and categorize all the species and accessions of *Trifolium* in what is now known as the Margot Forde Germplasm Center at Grasslands. Always on the lookout for clovers, Dr. Taylor made numerous "side trip" collections while traveling and/or attending meetings. An example of this was a side trip during a Southern Pasture and Forage Crop Improvement Conference meeting at Overton, TX where Taylor, Quesenberry, G. Ray Smith, and Gary Pederson identified and collected seed of *T. reflexum* L. just along the fence line of Hopewell Cemetery near Overton (PI 650856). He maintained a large worldwide network of scientists interested in clovers with whom he exchanged *Trifolium* germplasm. This networking resulted in continual increases in the number of species in the UK collection. Because of the diversity in the UK collection, he was designated by the NPGS as the *Trifolium* curator. After his passing, the seed samples in the UK collection were transferred to two sites: the Southern Regional Plant Introduction Station in Griffin, GA (annual species) and Western Regional Plant Introduction Station in Pullman, WA (perennial species). Current GRIN records show that Dr. Taylor contributed over 810 accessions to the NPGS that included 16 unique species and 40 unique accessions.

A particular interest of his was collection and maintenance of North American *Trifolium* species. He, Ken Quesenberry, and Daniel Boone (co-author of this paper - not the late 1700s Daniel Boone) were involved in trips that identified new locations for Eastern North American species, specifically *T. calcaricum* J. L. Collins & T. F. Wieboldt, *T. carolinianum* Miichx., *T. reflexum, T. stoloniferum* Muhlenberg, and *T. virginicum* Small ex Small & Vail. One particularly fascinating collection trip and location is a site in SW Virginia identified for the presence of *T. calcaricum*. Dr. Taylor and Daniel Boone traveled west to east (opposite track of that earlier Daniel Boone) through the Cumberland Gap (now US highway 25E) to access this site and made a collection that is now a part of the NPGS holdings. He investigated the morphology and crossing relationships in this group of species and showed that all these species are strongly genetically isolated with no interspecific hybridization observed (Taylor, et al., 1994).

Drs. Taylor, Quesenberry and Warren Williams from New Zealand collected over 25 annual and perennial species (total of over 75 accessions) in California, Oregon, and Washington in 1994. These western USA collection expeditions resulted in the addition of at least 20 new species to the USDA NPGS GRIN *Trilofium* collection. One of the widespread species collected in these efforts was *T. wormskioldii* Lehm. This rhizomatous clover was collected from sandy coastal dune sites within a few m of the Pacific Ocean surf, from volcanic rocky sites along the sea coast, but also from inland mountainous meadows along small streamlets. It is postulated that this species could have genes for salt tolerance and adaptation to wet soil sites. K. Quesenberry has identified locations for this species along the Pacific coast from S of Monterey, CA to N of Seattle, WA, suggesting variability in adaptation to different daylengths. An example of Dr. Taylor's tenacity in locating and collecting rare species native to western North America is his trip with Walter Graves to Baja California, Mexico in 2000 to collect *T. wigginsii* Gillett. This species is endemic only to a limited area in Baja California, Mexico.

The Genus Trifolium

The majority of species in the genus *Trilofium* are native to Europe and western Asia with a small number in eastern Asia, but there are also concentrations of species in Africa and in western North America with a limited number of species native to eastern North America and South America (Table 1). Numerous partial taxonomic and phylogenetic treatments of the genus have been presented, but three complete treatments of the genus will be considered in this paper. These are: (1) 'The Genus *Trifolium*' (Zohary and Heller, 1984), (2) 'The World of Clovers' (Gillet & Taylor, 2001), and (3) 'Molecular Phylogenetics of the Clover Genus' (Ellison, et al., 2006). Depending on whether certain binominals are accepted as independent species vs. a synonym of another species, the genus consists of ± 250 species (Table 2). The subgenera and sections of the genus proposed by Ellison, et al (2006) will be used in classifying the species in this paper.

Base chromosome numbers and ploidy levels in *Trifolium* are more diverse than any other genera of herbaceous forage legumes (Gillett and Taylor, 2001). The base chromosome number of n = 8 (2n = 16) is the most common in the genus - 155 species, followed by n = 7 (2n = 14) - 15 species, n=6 (2n = 12) - two species, and n = 5 (2n =10) - three species (Table 3). One species is reported as a tetraploid of n =7 (2n = 4x = 28). All other polyploids are multiples of n = 8, including six reported as tetraploid (2n = 4x = 32), two reported as hexaploid (2n = 6x = 48), and one species each at 2n = 64, 80, 96 and 128. There are 18 species with variable chromosome number reports and 51 species with no reported chromosome numbers (Table 3). The mechanisms of chromosome number evolution in *Trifolium* are not understood but would seem to be a fertile area for modern DNA sequencing research.

Some interesting associations between chromosome numbers and other species attributes can be identified. All species in section *Involucrarium*, found only in North and South America, are n = 8, although nine have reports of polyploidy. All species in the small sections, *Glycyrrhizum* (two species), *Lupinaster* (three species), and *Paramesus* (two species) are n = 8. All species with published chromosome numbers in sections *Trifoliastrum* (nine species) and *Vesicastrum* (53 species) are also n = 8. Section *Trifolium* contains species of n = 5, 6, 7, and 8 and is certainly the most diverse taxonomic section. Section *Trichocephalum* contains only annual species (nine) of n = 6, 7, and 8. All known annual species except for *T. dubium* Sibth. 2n = 28 and *T. dichotomum* Hook. & Arn. 2n = 32 are diploid, but these annuals include n = 5, 6, 7, and 8. All perennial species with published chromosome numbers are n = 8, except for *T. pratense* L. and *T. trichocephalum*, both at 2n = 14. All the perennial polyploids are n = 8.

The diversity in seed dispersal morphology and methodology in *Trifolium* is another fascinating feature of the clovers. In some species the calyx throat is open and the legume very papery resulting in seed shattering. Others have long calyx teeth that can adhere to animal hair or human clothing and function as dispersal mechanisms (*T. stellatum* L.). Another dispersal mechanism is the evolution of a soft feathery calyx resulting in a "puffball" type mature head (*T.*

pauciflorum D'Urv, and *T. batmanicum* Katzn.) that blows in the wind. With the species *T. cherleri* L. the entire head separates with bracts from the peduncle to allow the flattened head to disperse. The species *T. clusii* Godr. & Gren. and *T. tomenosum* L. have inflated calyxes that allow individual mature florets to blow around in the wind. A particular unique dispersal form are the species *T. subterraneum* L. and *T. israeliticum* D. Zoh & Katzn. which form "burs" that bury seed into the top layers of the soil. Perhaps the most unusual floral and seed evolution is the species *T. polymorphum* Poir. Ex Lam. & Pior., which has two different morphologies of flowers. The most prominent morph is an upright compound head with florets similar to *T. repens* L. These aerial flowers have been shown to be primarily cross pollinated (Real, et al., 2007). The second morph is one to three florets that arise at the axis of the stolon and petiole and form a short "peg" like structure that buries a one to two seed pod in the upper layer of the soil and these flowers are self-pollinated (Fig 1).

Root morphology among *Trifolium* species covers much of the range of forms common among herbaceous higher plants. Most annual species have typical spreading fibrous root systems. Some perennial species, e.g. *T. pratense,* are tap rooted with persistent crowns from which yearly regrowth occurs. Other perennial species, e.g. *T. eriocephalum* Nutt., *T. douglasii* House, and *T. plumosum* Dougl. have a large swollen tap root that functions as an energy storage organ for survival during long dry summers and provides energy for regrowth in the following spring. Other species such as *T. repens*, T. *polymorphum,* and *T. fragiferum* L., have an abundance of stolon growth with rooting where each node contacts the soil forming a turf type mat of roots. There are other strongly perennial species that have extensive rhizomatous root systems, e.g. *T. alpestre* L., *T. ambiguum* M.B., *T. medium* L., *T. ochroleucum* Huds., and *T. pannonicum* Jacq. In native sites these species form clusters of large clonal groups that can often be distinguished in field collecting of germplasm. Other species such as *T. owyheense* Gilkey have evolved root systems to persist in very shallow rocky soils in areas like SW Oregon with very low annual rainfall (Table 3).

The North American distribution of *Trifolium* species is of interest in that it is bi-modal with a large group of species (56) native to states from the Rocky Mountains west to the Pacific, with the states of California, Oregon, and Washington having 35, 32, and 20 native species, respectively. But, then there is a large gap of states across the Great Plains with no native species. And then there is a small group of seven species (*T. bejariense* Moric., *T. calcaricum, , T. carolinianum, T. kentuckiense* Chapel & Vincent, *T. reflexum*., *T. stoloniferum,* and *T. virginicum*) native to the central and southeastern US. All these seven species except *T. carolinianum* are moderately to very rare in current distribution. Some of these species (*T. bejariense, T. calcaricum,* and *T. kentuckiense*) are only known in current active seed collections from two or three sites.

Only about 16 species are cultivated as forage crops (Gillet and Taylor, 2001), but numerous others contribute to native pasture and rangeland forage for grazing animals. The senior author has observed mountain meadows containing *T.alpestre, T. heldreichianum* (Gibelli & Belli) Hausskn., *T. medium, and T. ochroleucum* being hand harvested for hay in south central Bulgaria. In the southeastern USA some of the annual clover species such as low hop clover (*T. campestre* Schreb.), crimson clover (*T. incarnatum* L.), and ball clover (*T. nigrescens* Viv.) have been used by state Departments of Transportation in reseeding roadsides after construction. Particularly hop clover and ball clover have escaped from these types of plantings and can be found in the spring of the year in low maintenance areas and borders of shaded areas.

The two most widely cultivated and researched clover species are white and red clover. Each of these will be the subject of full papers in this symposium. The primary annual species in the genus that are used in cultivated agriculture will also be discussed in an additional paper. These main cultivated species have been the subject of research publications for over 100 years. With the resurgence of interest in sustainable agronomic practices like cover crops and conservation tillage applications during the past decade, there is a renewed interest in the use of both annual and perennial clovers either as pure stands or in mixtures with grasses. An example of this is the research that has been conducted at the University of Wisconsin Agronomy Department on intercropping kura clover (*T. ambiguum* M. Bieb.) with maize (*Zea mays* L) (Sawyer, et al., 2010). Numerous research reports over the past decade have highlighted the utility of both annual and perennial cultivated clovers as cover crops in rotation with maize, small grain, and other high value row crops.

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Figure 1. *Trifolium polymorphum* Ex Lam. & Pior. showing upright aerial flowers resemblimg those of *T. repens* L. and below ground seed pods from basal flowers. Photo courtesy Dr. Carlos Acuna.

Table 1. Geographic distribution of Trifolium spp in USDA NPGS collections			
Continent/Zone	# of species	# in GRIN	# not collected
E. North America	7	6	1
W. North America	56	47	9
South America	11	5	6
Africa	41	26	15
Eurasia	141	113	29
Total	256	197	59

Table 2. Taxonomic sections of species in the Genus *Trifolium* based on various sources.

Section	Ellison (218) + WOC[1]	Quesenberry-Zohary[2]	Ellison-Table[3]	GRIN Taxonomy[4]
Chronosemium	17	20	20	19
Glycyrrhizum	2	2	2	2
Involucrarium	66	73	72	67
Lupinaster	3	3	3	3
Paramesus	2	2	2	2
Trichocephalum	8	9	9	9
Trifoliastrum	14	20	20	20
Trifolium	59	74	73	73
Vesicastrum	47	53	54	51
Unclassified	23	0	0	0
Total	241	256	255	246

[1] Ellison et al., 2006 - Gillet and Taylor, 2001; [2] Zohary and Heller, 1984; [3] Ellison et al., 2006; [4] USDA ARS NPGS GRIN-Global/Taxonomy (https://npgsweb.ars-grin.gov/gringlobal)

Table 3. Classifications of Trifolium species by various attributes					
Growth habit	158 annuals	95 perennials	3 short lived perennials		
Chromosome #	3 - 2n = 10	2 - 2n = 12	15 - 2n = 14	155 - 2n = 16	
Chromosome #	1 - 2n = 28	6 - 2n = 32	5 - higher ploidy	18 - mixed reports	51 - no reports
Breeding system	88 - self-compatible	73 - self-incompatible	94 - no reports		
Root system	209 - tap rooted*	19 - stoloniferous	27 - rhizomatous		
Flower color	69 - white	163 - red/pink/purple	20 - yellow	3 - no reports	
Cultivated	11 - annuals	5 - perennials			
*Tap rooted includes fibrous rooted annuals, perennials with primary tap root plus branching, and perennials with mainly large tap root.					