## **Previous Research – taxonomic monography**

*Leucaena* (Leguminosae – Mimosoideae) is a small genus of 17 diploid and 5 tetraploid species, six infraspecific taxa, and two named hybrids. They are small to medium-sized trees that grow in seasonally dry tropical forests of Mexico, Central America and northern South America. Previous work on *Leucaena* combined species delimitation and description with systematic analysis of species and generic relationships. This research culminated in two major publications - a *Taxonomic Monograph* of the genus (Hughes, C.E. 1998. Systematic Botany Monographs 55), and a *Genetic Resources Handbook* (Hughes, C.E. 1998. Tropical Forestry paper 37, Oxford Forestry Institute) which documents the economic importance of the genus.

The economic importance of *Leucaena* species, and especially *L. leucocephala* for the production of livestock fodder, green manure, small wood products and for soil conservation is well known and widely documented. *Leucaena* species are cultivated pantropically as forage and wood crops, and *L. leucocephala* is widely naturalized and weedy in many tropical countries. In Mexico, 13 species of *Leucaena* are used as minor food plants and have undergone a complex process of incipient indigenous domestication. Several putative hybrids in *Leucaena* appear to have arisen spontaneously following juxtaposition of previously isolated species in cultivation in informal orchards in south-central Mexico (Hughes et al., 2007).



## **Current Research - Gene trees, species trees and reticulate evolution**

Despite the high frequency of hybridization and polyploidy in plants, significant challenges remain to disentangle reticulation from divergence in plant phylogenies. In collaboration with Donovan Bailey (New Mexico State University) we are investigating these questions for Leucaena using a gene tree approach. Studies of reticulation demand multiple, densely sampled, highly resolved and robustly supported biparentally inherited gene trees. Much of the work on Leucaena has been devoted to tackling problems posed by lack of informative variation in conventional DNA sequence loci and the occurrence of nrDNA pseudogenes (Hughes et al., 2002; Bailey et al., 2003, 2004) - issues that hinder reconstruction of robust, well-resolved species-level phylogenies for plants. This has involved one of the first studies to thoroughly explore the impacts of nrDNA polymorphism and non-functional pseudogene sequences on phylogeny reconstruction in plants (Hughes et al., 2002; Bailey et al, 2003) and development of a powerful method for identifying pseudogene sequences using phylogenetic trees and bootstrap hypothesis testing (Bailey et al., 2003). We showed that pseudogene sequences are common in nrDNA datasets, which dominate plant species-level phylogenetics, and that their detection is critical for accurate phylogeny reconstruction. To get around the problem of lack of phylogenetic resolution, we developed a novel SCAR-based technique to identify more rapidlyevolving, biparentally-inherited, nuclear DNA sequence loci that can generate gene trees with the required resolution and support (Bailey et al., 2004). Using one of these loci a number of putative hybrids have been investigated (Hughes et al., 2007).



The evolutionary dynamics of polyploidy in Leucaena (from Govindarajulu et al., 2011b).

Comprehensive sequence data sets for a larger set of SCAR-based nuclear DNA sequence loci have been generated by Rajanikanth Govindarajulu, a PhD student working with <u>Donovan Bailey</u> (Govindarajulu et al., 2011a, 2011b). These data provided the basis for a well-resolved and robustly supported diploid phylogeny, that suggests a predominant pattern of allopatric diploid species divergence (Govindarajulu et al., 2011a). Alongside population genetic analyses of AFLP data, these new analyses also provide evidence for recognition of two additional cryptic species, *L. zacapana* and *L. cruziana* formally recognized by Govindarajulu et al. (2011a). These comprehensively sampled multilocus nuclear DNA sequence data sets, and a novel 'spene' tree method, provide evidence for four independent allopolyploidization events and the likely diploid parentages of the five known tetraploid *Leucaena* species (Govindarajulu et al., 2011b). A more comprehensive picture of the complex evolutionary dynamics of polyploidy is emerging. This has involved paleaopolyploidization, diploidization, allopatric diploid species divergence and recent allopolyploidization (Govindarajulu et al. 2011a, 2011b), much of the latter potentially precipitated by spontaneous hybridization of species brought into artificial sympatry by human cultivation, has now been established (Hughes et al., 2007).