

## A model for floral color inheritance in *Leucaena* (Leguminosae)

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### ABSTRACT

A population of 513 hybrids of *Leucaena leucocephala* (4x) and *L. diversifolia* ssp. *diversifolia* (4x), that is being analyzed for several morphological and agronomical characteristics, aiming at selecting cold tolerant plants, was also analyzed for floral color. A model of additive dominant inheritance (pink color dominant over white color, or presence versus absence of pigment, and the number of dominant alleles present determining the intensity of the pink color) is proposed. The action of two independent locus sets is also suggested, one for the male (with two main structural genes) and another for the female floral organs (one main structural gene). It is suggested that an undetermined number of regulatory genes affect color expression.

### INTRODUCTION

Color is undoubtedly one of the most outstanding features of many flowers, not only because of its role in attracting pollinators, but also for the beauty it brings to nature and human life. Flower color should be considered an important component of flower development. It has been used for genetic studies since Mendel (Martin and Gerats, 1993). The biochemistry of pigment production

has been extensively studied. It is known that flower color is formed mainly by anthocyanins, but actual color can be affected by other pigments, metal ions and vacuolar pH (Martin and Gerats, 1993).

Mendel determined that flower color was monogenically inherited in sweetpea, but mutations have been described that affect pigment biosynthesis and distribution in many flowering plant species. Pigmentation is normally discrete within tissues and organs and determined by structural and regulatory genes (Martin and Gerats, 1993).

*Leucaena* Benth is one of the most well-known and widespread genera of nitrogen-fixing multipurpose trees; it has great economic and social importance in the tropics. The main obstacles to greater use of *Leucaena* are related to tolerance to environmental stress, such as cold, frost, acidic soils, pests and diseases (Shelton *et al.*, 1995). Classical taxonomy, cytogenetic, isozyme, and DNA analyses are some of the approaches that have been used to clarify the taxonomy and evolution of this Central American genus. Hughes (1993) described 17 species of *Leucaena*, but more recent data recognize 22 species, plus intraspecific and hybrid taxa also with new nomenclature. (Hughes, 1988 and in press). Some of the species are endemic, but others are widely distributed beyond their centers of origin and form morphologically variable complexes (Hughes, 1993; Zárate, 1994). Isozyme analysis has disclosed significant variation between and within natural populations (Harris *et al.*, 1994a, Chamberlain *et al.*, 1996). The extremely narrow genetic base of the worldwide cultivated species *L. leucocephala* and *L. diversifolia* has been extensively described and may explain the susceptibility of cultivated *Leucaena* to the psyllid *Heteropsylla cubana* (Hughes, 1993). Chromosome counts have been made for some species but not for many accessions. Diploid ( $2n = 52$  and  $56$ ) and tetraploid ( $2n = 104$  and  $112$ ) species have been identified, but it is accepted that the genera is paleopolyploid (González *et al.*, 1967; Freitas *et al.*, 1988; Pan and Brewbaker, 1988; Palomino *et al.*, 1995). DNA techniques have given new insights into *Leucaena* systematics and evolution (Harris *et al.*, 1994b; Harris, 1995).

Most *Leucaena* species have whitish-yellowish inflorescences, with the exception of *L. gregii*, *L. retusa* (both with bright yellow flowers) (Hughes, 1993), *L. diversifolia* ssp. *diversifolia* (4x), *L. diversifolia* ssp. *stenocarpa* (2x) (different species according to DNA analysis by Harris (1995) and taxonomic studies by Hughes (in press)) *L. confertiflora* and *L. esculenta* ssp. *paniculata* (*L. pallida*) (Harris, 1995; Hughes, 1988 and in press). Pink or yellow pigments are present in the male (stamens-filaments) and female (style-stigma) organs. The petals and bracteate sepals are always greenish-white.

Pan (1985) analyzed intraspecific diploid *L. diversifolia* hybrids and observed variation in what he called flower color tonality from dark pink to light pink. He suggested that heterozygotes for the gene or genes controlling this characteristic should occur in natural populations. He also reported that pigment was seen in one or more of the anthers, styles, and /or filaments.

Freitas *et al.* (1991a) studied hybrids between tetraploid *L. leucocephala* (white flowers) and tetraploid *L. diversifolia* ssp.

*diversifolia* (pink flowers) and also found variation in pink tonality and pigment distribution among floral parts in 49.7% of pink flower individuals. The final pink tonality was determined by which floral parts were pigmented (anthers, filaments, styles) and by the amount of pigment present in each one. These results suggested the combined action of structural and regulatory genes.

The objective of our study was to examine progenies of the population studied by Freitas *et al.* (1991a,b, 1995), and to propose a model for inheritance of floral color in *Leucaena*.

## MATERIAL AND METHODS

The original crosses between the two  $2n = 104$  species, *L. leucocephala* and *L. diversifolia* ssp. *diversifolia*, were made by Dr. E.M. Hutton at CIAT, Cali, Colômbia. A hybrid population, formed by 255 ( $2n = 104$ ) trees from 51 mother-individuals, was established at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, Brazil. These plants were evaluated for cold and frost tolerance (Freitas *et al.* 1991a,b, 1995). Twenty of the most vigorous and productive trees were selected. A population of 600 trees was established, from freely pollinated seeds, forming 20 families, each one composed of 30 individuals from each of the 20 previously selected trees; 513 flourished and were analyzed. These trees were and continue to be evaluated for several morphological, physiological and agronomical characteristics (Simioni, 1996).

Floral color parts were evaluated under a stereomicroscope in 10 young, opened flowers from each of the 513 trees. Color and pink shade of styles (stigmas were almost always white), stamens, and filaments were recorded.

Data were organized into phenotypic classes, regarding the color of stamens, filaments and styles ([Table I](#)). The results were compared to the general flower color of the 20 seed donor trees (separate data for male and female organs are not available for these plants) ([Table II](#)).

**Table I** - Phenotypic classes for floral color and number of plants in each class for the *Leucaena hybrid* population studied.

Phenotypic class	Number of plants
White filament, pink anther, white style	12
White filament, pink anther, very light pink style	7
White filament, pink anther, light pink style	19
White filament, pink anther, dark pink style	30
White filament, pink anther, very dark pink style	18
White filament, white anther, white style	279
White filament, white anther, very light pink style	60
White filament, white anther, light pink style	38
White filament, white anther, dark pink style	29
White filament, white anther, very dark pink style	10
White and pink filament, pink anther, very light pink style	1
White and pink filament, pink anther, dark pink style	2
White and pink filament, pink anther, very dark pink style	8
Total	513

**Table II** - Number of progeny plants with different flower colors, compared with the original mother plants (seed donors) (see text).

Mother-plants	Progeny		
	Family number	Flower color	White flowers
1.1	white	10	12
6.5	white	7	16
9.5	white	11	16
19.3	pink	26	1
23.1	pink	21	3
23.2	pink	0	27
24.5	pink	1	25
26.3	pink	1	24
35.3	white	27	1
46.2	white	3	25
46.4	white	17	12
46.5	white	10	17
48.2	white	13	14
49.1	white	7	22
50.1	white	25	2
50.3	white	17	4
50.4	white	9	7
50.5	white	25	3
51.1	white	30	0
51.3	pink	19	3
Total		279	234

## RESULTS AND DISCUSSION

The proportion of individuals with white flowers and with pink flowers was nearly 1:1 (Table II). Data suggested independent inheritance of the masculine (anthers and filaments) and of the feminine (styles) organs. Data from Table II can be interpreted relative to the occurrence of heterozygosity and cross pollination in the hybrid population. *L. leucocephala* was described as autocompatible and selfpollinated by Brewbaker (1983) and *L. diversifolia* ssp. *diversifolia* was described as autoincompatible by Pan (1985). Hybrids between these two taxa are easily obtained. Cross pollination occurs in hybrid populations at undetermined ratios. The two species are tetraploid ( $2n = 4x = 104$ ), and existing information suggests an allotetraploid origin of both species

(Hughes, in press). Since cp DNA analysis indicated that *L. pulverulenta* may be a common ancestor to both *taxa* (Harris *et al.*, 1994b), they can be considered as segmental allotetraploids.

We propose the existence of two independent structural locus sets: one set, with two main structural genes, for anther-filaments, and another set, with one main structural gene, for styles. Two genes are proposed for male organs because, anthers can be white and filaments pink or vice-versa ([Table I](#)). At each locus, the presence of pink pigment (P) would be dominant over the absence of pigment (W). The expression would be additive and more P alleles would increase pink shade. Four recessive alleles would determine white color (no pigment) and four dominant alleles would lead to a very strong pink color. All combinations of W and P would determine the different pink shades: WWWP- very-light pink, WWPP- light pink, WPPP- strong pink. The mother trees would form WW, WP or PP gametes, depending on their original constitution ([Table II](#)). Because male gametes could also be of these three types, random crosspollination in this hybrid cross compatible population would explain the existence of several phenotypic classes within the descendants of one original mother-plant. Separate locus sets for both male and female floral parts are expected if we consider the divergent development and functions of these floral organs. It would be much more complicated to suppose that a single locus determines the color of all floral parts. Our data on pink shade variation ([Table I](#)) strongly support the existence of regulatory gene(s) determining the amount of pigment formed by the action of each structural allele.

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## RESUMO

Uma população de 513 híbridos entre as espécies tetraplóides ( $2n = 104$ ) *L. leucocephala* e *L. diversifolia* ssp. *diversifolia*, que estão sendo analisados quanto a diversas características agrônômicas e morfológicas, com o objetivo de selecionar plantas tolerantes ao frio, foi também analisada considerando a cor das partes florais. É proposto um modelo de herança dominante e aditiva (rosa dominante sobre branco, ou presença versus ausência de pigmento e o número de alelos dominantes determinando a intensidade da cor rosa). Também sugere-se a ação de dois conjuntos independentes de locos, um conjunto para os órgãos masculinos (com dois genes estruturais principais) e o outro conjunto para os órgãos femininos (um gene estrutural principal), e que a expressão da cor é afetada por genes regulatórios.

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