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Genetic diversity and structure of the endemic *Caesalpinia hintonii* complex (Leguminosae: Caesalpinioideae) in Mexico

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Abstract. The *Caesalpinia hintonii* complex is formed by five endemic species (*C. hintonii*, *C. laxa*, *C. macvaughii*, *C. melanadenia* and *C. epifanioi*) occurring in central Mexico. This species complex is under incipient genetic divergence as by-product of local adaptations in reproductive and morphological traits to different habitats. We estimate the genetic variation and structure of populations of this species complex to assess the extent of genetic differentiation among populations and related species along its geographic distribution. Estimations of genetic diversity and structure were done based on ten enzymes and 18 loci. Mean number of alleles per locus ranged from 1.5 to 1.9. Polymorphic loci ranged from 42.1 to 68.4. Observed (H_o: range 0.191–0.275) and expected (H_e: range 0.205–0.317) heterozygosities in this complex were higher compared with other endemic and legume species. Nei's genetic diversity estimates showed that most genetic variation was found within (H_S = 0.325) rather than among populations (D_{ST} = 0.085). Populations of the species *C. hintonii* showed a considerable genetic differentiation (F_{ST} = 0.207). The results of genetic diversity and structure within and among populations are in accord with the great morphological differentiation described for this species complex.

Key words: *Caesalpinia hintonii* complex, central Mexico, endemic species, genetic structure, heterozygosity, Leguminosae.

The Caesalpinia assemblage in the tribe Caesalpinieae, as circumscribed by Polhill and Vidal (1981), comprises 16 genera of trees and shrubs or vines, distinguished primarily by having leaf axes adaxially ridged, and mostly zygomorphic flowers with a modified abaxial sepal and stamens arranged towards the gynoecium (Kantz and Tucker 1994). Predominantly found in seasonal forests in semiarid and arid environments, the plants of this group are characterized by presenting diverse defense systems based on thorns, prickles and glandular hairs. Within this assemblage, the genus *Caesalpinia* differentiates from all the genera by an array of morphological traits, specially in its reproductive structures and high number of species (ca. 130), of these, 45 species occurring in Mexico (Sousa and Delgado-Salinas 1993).

Therefore, the genus *Caesalpinia* represents a remarkably successful lineage of morphologically distinct species although intraspecific

geographic variability often transcends interspecific variability in this group, leading to difficulties in deciding the taxonomic status of closely related populations. Resolution of the taxonomic difficulties created by such patterns of cladogenesis requires the use of molecular markers to define genetically isolated groups and to assess the distribution of genetic and morphological variability within and between species.

One group of *Caesalpinia* in which the species level taxonomy has posed continuing problems is the so-called *C. hintonii* group or complex (Contreras 1991, Lewis 1998). This complex includes *C. hintonii* Sandw., *C. epifanioi* J.L. Contr., *C. macvaughii* J.L. Contr. & G. P. Lewis, *C. laxa* Benth., and *C. melanadenia* (Rose) Standl., and has proved to be monophyletic (S. Sotuyo et al. unpubl. data). All taxa are confined to the Sierra Madre del Sur Morphotectonic Province, in the Río Balsas Depression or Basin and to the neighbor Tehuacán – Cuicatlán – Quiotepec Depression subprovinces (Ferrusquía-Villafranca 1993), in the states of Puebla, Oaxaca, Guerrero and Michoacán (central Mexico). This region is characterized by different types of vegetation, all related to a well-defined dry season, which is inhabited by a considerable number of endemic groups of plants within Burseraceae (Becerra and Venable 1999), Commelinaceae (Hunt 1993) and Leguminosae (Sousa and Soto 1987, Sousa and Delgado-Salinas 1993, Lewis 1998). Likewise, the region has been considered a center of diversification for various animal taxa, like in different groups of mammals (Fa and Morales 1993). Moreover, Sousa and Delgado-Salinas (1993) have suggested recent and active speciation in hot-dry areas of Mexico for *Caesalpinia*, particularly in the Balsas basin region. Therefore, diversification in several groups in this region is common and even within populations, like the one reported for the species *C. hintonii*, where three forms or morphs have been described by Contreras (1991) and later on, recognized by Lewis (1998) in his taxonomic revision of the *Poincianella – Erythrostemon* group.

Additionally, this species complex offers an interesting opportunity to relate the extent of genetic diversity between congeneric species with different extent of geographic distribution and provides the possibility of evaluating the general trends on genetic diversity of rare versus common plant species. In contrast to previous reports, a recent evaluation suggests that rare and widespread congeneric species are highly correlated in terms of genetic variation (Gitzendanner and Soltis 2000). Thus, considering the historical constraints and the role of the evolutionary history of related species may help to understand better the dynamics of population genetic structure and variation of rare species.

Since the *C. hintonii* complex group has been suggested to be under present day speciation and other source of evidence is needed to determine how many species or infraspecific taxa should be recognized (Lewis 1994, 1998), we decided to conduct a study with the following objectives: (1) to asses the population genetic diversity and structure to clarify the genetic relationships between taxa included in the *C. hintonii* complex, and (2) to address variation in the genetic diversity between endemic conspecific taxa with different extent of geographic distribution at a regional scale.

Materials and methods

The species complex. Species descriptions and flower drawings (Fig. 1) were compiled for all taxa from herbarium specimens (MEXU) and from Contreras (1991) and Lewis (1998), except for *C. laxa* Bentham, that was not included in this study because of our inability to find equal number of plants to be sampled. All the species studied are diploid (Ibarra-González 2002) and allogamous (Lewis 1994, 1998).

Caesalpinia hintonii plants are medium-sized trees, 2–7 m tall, with greyish-white, exfoliating, rarely corky bark. Leaflets are arranged in 3–10 pairs per pinnae, with 3–6 pinnae pairs per leaf, and a terminal pinna. The inflorescence can be displayed in lax, erect or pendent axes, with multiflowered racemes on glandular rachis (pedicels occasionally densely stipitate-glandular). Corollas

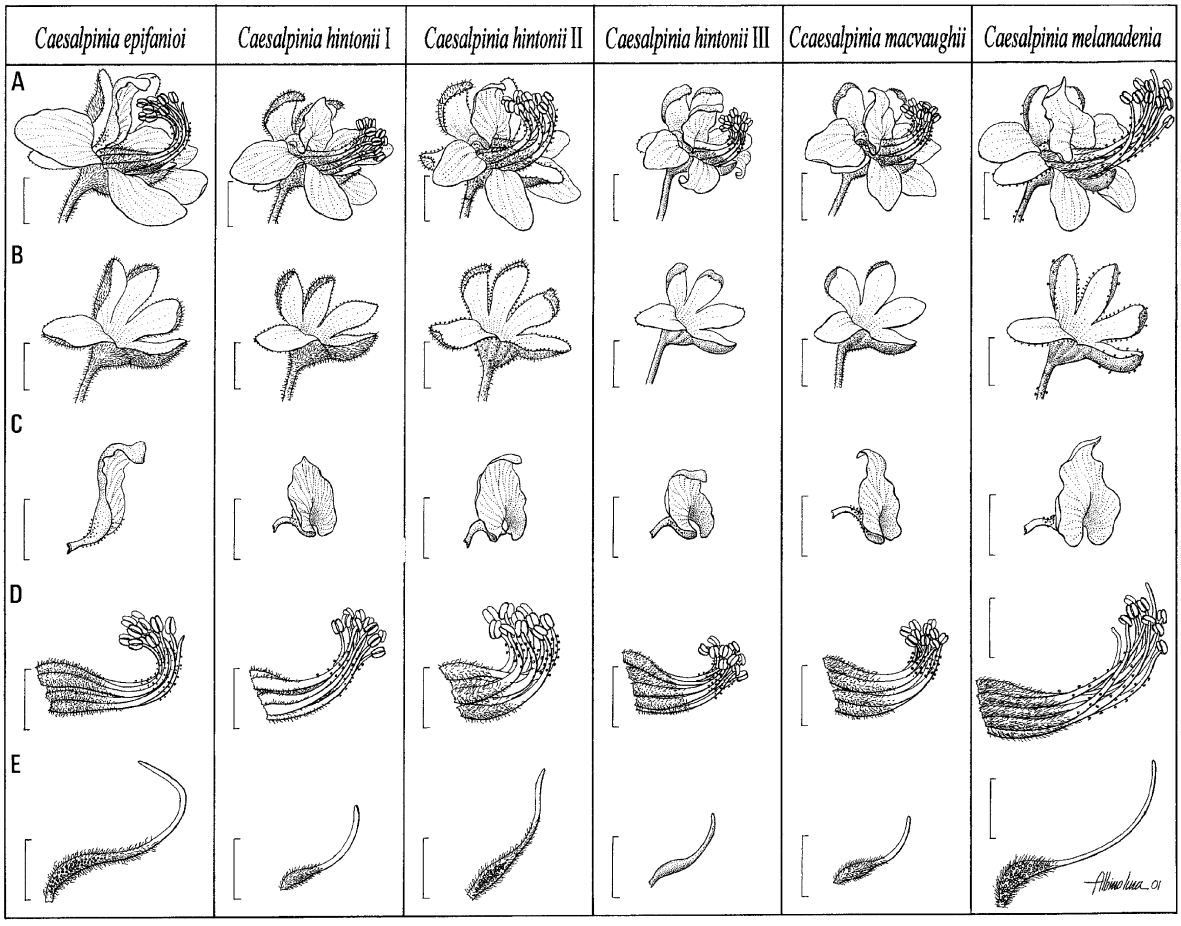

Fig. 1. Floral comparison between the members of the *Caesalpinia hintonii* complex, *C. epifanioi*, *C. hintonii* represented by three morphs (morph I, morph II, morph III), *C. macvaughii*, and *C. melanadenia*. **A** Flowers, side view. **B** Calyxes, without other flower parts. **C** Standard petal. **D** Androecium. **E** Gynoecium. Scale bar equals 4 mm

show yellowish sepals and red-pink petals. Distributed mainly on the Río Balsas Depression in the states of Puebla, Guerrero and Michoacán, in a variety of habitats, including dry-deciduous forests, at low to mid elevations (150–1300 m). Three morphs or forms can be recognized in this species. The first morph (morph I) is distinguished by erect or ascending inflorescences, with abundant indumentum; with flowers that are supported by appressed-ascending pedicels, non-resupinated. The fruit has lime green, cupular or annular glands in its surface. This form is represented here by plants from the localities Valerio Trujano and Tlayahualco (Guerrero) and from an isolated population in the eastern portion of Río Balsas Basin, in Tehuitzingo (Puebla). The other two forms are characterized by have long-curved pendulous inflorescences, with slender pedicels that can be bent or twisted so the flowers appear resupinate. The second morph or morph II (from Zicuítaro) has indumentum composed of simple hairs or mixed with glands; larger leaflets and larger scarlet flowers, these supported by articulated pedicels. The legume is covered with abundant red, stipitate pixie-cup glands. The third form or morph III (from Infiernillo) is glabrous with glands restricted only to the floral structures; it presents smaller leaves with fewer leaflets and smaller flowers with yellowish sepals and salmon pink petals. The pedicels are articulated at or below their middle part. The legume is completely glabrous and only occasionally presents stipitate glands on both surfaces. The known geographic distributions of the three forms are allopatric, with only a small

region of sympatry of morphs II and III, in the region of Infiernillo.

Caesalpinia macvaughii is a shrub to medium-sized tree, 2–8 m tall, with pruinose- grey exfoliating bark. Leaves with caducous stipules, and leaflets disposed in 4–11 pairs per pinnae, 2–7 pinnae pairs per leaf, plus a terminal pinna. The margins of the leaflets are dotted with black glands. The inflorescence rachis is glabrous, displaying corollas with red pigmentation on the calyx and yellow flowers; the standard petal blade is ovate. The stamen filaments are curved, 6–10 mm long, flattened and densely villous at the base. This species occurs below 200 m in elevation, in dry deciduous forests, in the states of Michoacán and Guerrero.

Caesalpinia melanadenia is a multiple-stemmed shrub or small tree with contorted branches, 1–6.5 m tall. The bark of main stems is pale grey with smooth, pustular, and white lenticels. Leaves have stipules ovate and axes are covered with stipitate glands, sometimes densely so. Each leaf has 1–2 pinnae pairs per leaf, and a terminal pinna; leaflets are placed on 3–4 pairs per pinnae. The inflorescence rachis and pedicels are covered with stipitate glands. Racemes arise from short woody brachyblasts and have 10–20 flowers. The corolla has a red calyx and dark scarlet-pink petals. The standard petal blade is broadly triangular-hastate. The stamen filaments are 13–15 mm long. This species is distributed in Puebla and just over the border into Oaxaca, in mid to high elevations (800 to almost 2000 m), mainly in xeric shrublands.

Caesalpinia epifanioi is a shrub or tree, 2–4 m tall, with a bark grey and smooth. Leaves with stipules broadly ovate, and each leaf is composed of 1–2 pinnae placed in 1–2 opposite pairs, plus a terminal pinna. The leaflets are in 2–3 opposite pairs. The inflorescences are racemes on woody brachyblasts with several flowers; the corolla has a red calyx with yellow flowers. This species is known only from San Francisco Ozomatlán, state of Guerrero, at 500 m elevation.

Site locations of all sampled species and *C. hintonii* different morphological populations have been mapped on Fig. 2.

Isozyme electrophoresis. The numbers of genetic loci and alleles controlling the enzyme activity were inferred from the observed banding patterns and from data on quaternary structure. Bands of activity were assigned to loci that were numbered sequentially from the low anodal.

Isozyme analyses were carried out for five populations of *C. hintonii*, and one population of each of the following species: *C. epifanioi, C. macvaughii*, and *C. melanadenia* (Fig. 2). This

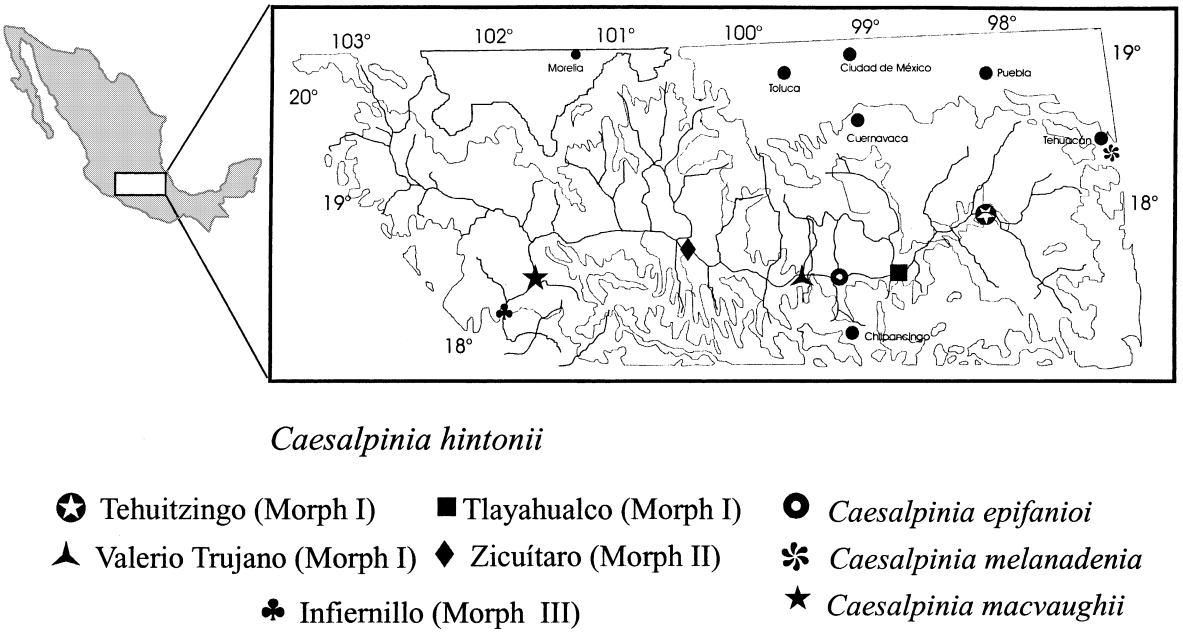

Fig. 2. Location of sampling sites in central Mexico of *Caesalpinia macvaughii*, *C. melanadenia*, *C. epifanioi* and of five populations of *C. hintonii*, representing the three studied morphs (morph I, morph II, morph III)

sampling collection represents the whole known distribution and populations of the *C. hintonii* complex except for *C. laxa*, which was not sampled for this study. However, preliminary phylogenetic analyses had proven that *C. laxa* could not be considered part of this complex of species (S. Sotuyo et al. unpubl. data). Fresh leaves were collected from plants growing in the field. Thirty (or less) individuals were sampled from each population. The tissues were crushed in 0.25 ml of a cold extraction buffer containing buffer YO (Yeh and O'Malley 1980) and buffer Veg II (Cheliak and Pitel 1984) mixture (3:1). The extract was absorbed into Whatman #12 filter paper wicks. Wicks were loaded into 12% starch gel. After electrophoresis, the gels were stained for isozymes by applying standard histochemical methods (Wendel and Weeden 1989) with modifications described by Sotuyo (1999). After screening 20 enzymes, ten had scorable bands representing 18 loci. System C (Stuber et al. 1988) resolved anodic peroxidase (APX - E.C. 1.11.1.7), catodic peroxidase (CPX - E.C. 1.11.1.7), esterase (EST - E.C. 3.1.1), leucine amino-peptidase (LAP - E.C. 3.4.1.1), menadione reductase (MNR - E.C. 1.6.99.2), and RUB (RUBISCO - 4.1.1.39). System Morfoline-Citrate (Wendel and Weeden 1989) resolved acid phosphatase (AcPH - E.C. 3.2.3.2), glucose-6-phosphate isomerase (GPI - E.C. 5.3.1.9), malate dehydrogenase (MDH - E.C. 1.1.1.37), and shikimate dehydrogenase (SDH - E.C. 1.1.1.25). Staining schedules were done following Soltis et al. (1983).

Bands of activity were assigned to loci that were numbered sequentially from the low anodal. The numbers of genetic loci and alleles controlling the enzyme activity were inferred from the observed banding patterns and from data on quaternary structure.

Data analysis. The Biosys-2 program (Swofford et al. 1997) was used to estimate the mean number of alleles per locus (A), the percentage of polymorphic loci (P), the observed (H_o) and the expected (H_e) heterozygosities under Hardy-Weinberg equilibrium. A chi-square test was used to test for statistical significance of the deviations between observed and expected heterozygosities under Hardy-Weinberg equilibrium.

Wright's (1921) inbreeding coefficient (F) was also calculated for each polymorphic locus and population; it measures the decrease in number of heterozygous plants due to non-random mating between individuals. The significance of deviations of F from zero was determined by the chi-square $\chi^2 = F^2\ N\ (k-1)$ test, with d.f. = [k (k−1)] / 2; where k = number of alleles in the locus and N = sample size (Li and Horovitz 1953).

Genetic diversity between populations was analyzed by using G-statistics (Nei 1973, 1987) defined by the formula $H_T = H_S + D_{ST}$ where H_T is the gene diversity in the total population, H_S is the average gene diversity within populations, and D_{ST}, is the average gene diversity among populations. D_{ST} was obtained by the difference $H_T - H_S$. The genetic differentiation between populations was obtained as $G_{ST} = D_{ST} / H_T$.

The population genetic structure within each species was analyzed using the procedure of Weir and Cockerham (1984) to calculate Wright F-statistics (1951). Mean and variance were estimated for each locus by jackknifing over populations and a summary value for each F-statistic by jackknifing over loci. To test whether the jackknifed means were significantly different from zero, a simple t-test were used. The 95 % confidence intervals (CI) for the summary values were obtained by bootstrapping over loci with 1000 iterations using the TFPGA (Tools for Populations Genetic Analyses) (Miller 1997).

Gene flow (Nm, the number of migrants per generation) was calculated using Slatkin's (1993) equation as follows: $Nm = [(1 / F_{ST}) - 1] / 4$. Nei's unbiased genetic distances (Nei 1978) were calculated and the values clustered using the unweighted pair-group mean method with arithmetic mean (UPGMA) (Sneath and Sokal 1973).

Results

A total of 18 loci were scored for ten enzymes surveyed; 15 of them were polymorphic in the *C. hintonii* species complex. The mean number of individuals scored per locus (N), mean number of alleles (A), the percentage of polymorphic loci (P), and the observed (H_o) and expected (H_e) heterozygosities are given in Table 1. Some alleles were species- or morph-specific: alleles 3 of *AcPH-1* and *EST-1* in *C. hintonii* (Infiernillo – morph III), allele 3 of *CPX-2* in *C. hintonii* (Tehuiztingo – morph I), allele 3 of *MNR-1* in *C. epifanioi*, and allele 2 of *EST-3* is shared by *C. macvaughii* and *C. epifanioi*.

Table 1. Genetic diversity parameters within populations of the *Caesalpinia hintonii* species complex in central Mexico. Mean number of individuals scored per locus (N), number of alleles per locus (A), percentage of loci polymorphic (P), observed heterozygosity (H_o) and expected heterozygosity (H_e). Standard deviations are in parentheses

Species Populations	N	A	P	H_o	H_e
Caesalpinia hintonii					
Valerio Trujano (morph I)	17	1.9 (0.17)	63.2	0.252 (0.082)	0.285 (0.056)
Tlayahualco (morph I)	15	1.9 (0.18)	63.2	0.267 (0.076)	0.263 (0.55)
Tehuitzingo (morph I)	15	1.8 (0.16)	57.9	0.261 (0.71)	0.251 (0.53)
Zicuítaro (morph II)	19	1.9 (0.17)	68.4	0.275 (0.074)	0.317 (0.055)
Infiernillo (morph III)	16	1.6 (0.17)	42.1	0.191 (0.073)	0.205 (0.056)
Mean	16.4	1.8 (0.2)	59.0	0.249 (0.055)	0.264 (0.055)
C. macvaughii	12	1.5 (0.1)	50.0	0.260 (0.107)	0.211 (0.066)
C. melanadenia	15	1.7 (0.1)	60.0	0.268 (0.086)	0.222 (0.052)
C. epifanioi	17	1.9 (0.2)	47.1	0.212 (0.073)	0.213 (0.059)

Genetic variation. The mean number of alleles per locus (A) within populations ranged from 1.6 to 1.9 in *C. hintonii*, and from 1.5 (*C. macvaughii*) to 1.9 (*C. epifanioi*) in the other species of this complex. The percentage of polymorphic loci per population of *C. hintonii* ranged from 42.1% (Infiernillo - morph III) to 68.4% (Zicuítaro – morph II) with a mean of 59.0%. The highest percentage of polymorphism was observed in *C. melanadenia* (60.0 %) and the lowest in *C. epifanioi* (47.1%) (Table 1). The observed heterozygosity (H_o) within the populations of *C. hintonii* ranged from 0.191 (morph III) to 0.275 (morph II), whereas the range for expected heterozygosity (H_e) was from 0.205 to 0.317 for the same populations. The mean over all populations was 0.249 for H_o and 0.264 for H_e, respectively. *Caesalpinia epifanioi* had the lowest value for H_o (0.212) and *C. macvaughii* for H_e (0.211). *Caesalpinia melanadenia* showed the highest value of H_o (0.268) among species (Table 1).

Fixation indices. The fixation index (F) was determined for each polymorphic locus (Table 2). From the 85 inbreeding coefficients calculated for all the species and populations of *C. hintonii* complex, 44 were positive and 41 negative. Of these, only 24 loci (28.2%) were positive and significantly different from zero (deficiency of heterozygotes), and 10 loci (11.8%) were negative and also significantly different from zero (excess of heterozygotes) (Table 2). Most of the loci (60.0%) did not differ significantly from zero and we can assume that they were in Hardy-Weinberg equilibrium.

On average, F values for populations of *C. hintonii* ranged from 0.013 (Infiernillo - morph III) to 0.291 (Valerio Trujano – morph I) indicating, in general, deficiency of heterozygotes. In the case of *C. macvaughii* (–0.148) and *C. melanadenia* (–0.075), the negative values indicate excess of heterozygotes. In *C. epifanioi*, any loci differed significantly from zero (Table 2).

Genetic diversity. The total genetic diversity was high ($H_T = 0.410$) within populations of *C. hintonii* species. The largest value of H_T recorded was 0.622, for the *AcPH-2* locus, whereas the lowest was 0.126, for *LAP-1*. Most genetic diversity was found within populations ($H_S = 0.325$). The largest estimate of H_S was recorded for locus *EST-1* ($H_S = 0.505$), and the smallest in *CPX-3* ($H_S = 0.096$) (Table 3).

Between population diversity (D_{ST}) was smaller ($D_{ST} = 0.085$). Estimates of genetic diversity within populations (H_S) were higher for almost all loci than those for genetic diversity among populations (D_{ST}). Among the populations, 20.7% of the genetic variation were found between populations, and the remaining 79.3% within populations. The

Table 2. Fixation indices (F) of polymorphic loci in the four species of *Caesalpinia*. CH, *C. hintonii*; CMC, *C. macvaughii*; CM, *C. melanadenia*; CE, *C. epifanioi*. *P < 0.01

Locus	CH					CMC	CM	CE
	Valerio Trujano (morph I)	Tlayahualco (morph I)	Tehuitzingo (morph I)	Zicuítaro (morph II)	Infiernillo (morph III)			
AcPH-1	1.00*	−0.549*	−0.336	−0.167	−0.030	−0.286	−0.208	–
AcPH-2	−0.356	−0.369	−0.235	−0.166	–	–	−0.176	–
APX-1	0.615	0.025	0.447	0.346	0.637*	1.00*	–	–
CPX1	−0.115	0.385	–	0.836*	0.287	–	0.33	0.138
CPX-2	–	–	0.421	–	–	–	–	–
CPX-3	–	−0.016	–	1.00*	–	–	–	−0.017
EST-1	0.43	−0.494*	−0.188	−0.518*	−0.825*	−0.867	–	–
EST-2	−0.438	0.289	−0.760*	0.206	−0.143	−0.667	−0.429	−0.132
EST-3	–	–	–	–	–	–	−0.392	−0.421
GOT-2	1.00*	–	−0.019	0.400	1.00*	–	0.712*	1.00
LAP-1	1.00*	0.519*	0.179	–	–	–	–	–
MDH-1	−0.867*	−0.651*	0.780*	−1.00*	−1.00*	−0.622	−1.00	−0.143
MDH-2	–	–	–	–	–	–	–	0.474
MDH-3		–	–	–	–	–	–	0.487
MNR-1	0.033	1.00*	0.100	−0.357	0.469*	–	−0.549	−0.109
PGI-1	1.00*	1.00*	0.538*	0.615*	1.00*	0.550*	1.00*	0.915
PGI-2	1.00*	–	0.480*	0917*	–	–	–	–
SDH-1	−0.517*	–	–	0.409*	–		−0.047	−0.733
Mean	0.291	0.103	0.117	0.210	0.013	−0.148	−0.075	0.132

estimated coefficient of genetic differentiation (G_{ST}) was 0.207 (Table 3).

Population differentiation. The F_{ST} estimates suggested that most genetic variation of the populations of *C. hintonii* studied is allocated between rather than within populations (Table 3). The summary value for all loci of F_{ST} was 0.207 and significantly different from zero ($P < 0.05$) (Table 3). Estimates of F_{IS} ranged from −0.876 to one (mean 0.031) with four loci significantly different from zero, revealing a deficiency in heterozygous individuals. However, the average across loci did not differ significantly from zero; their corresponding CI values did overlap with zero (Table 3).

F_{ST} values among the five populations of *C. hintonii* were very variable (0.005-0.629) with a mean of 0.207. Gene flow among these five populations of *C. hintonii* was almost one ($N_em = 0.957$).

Genetic relations. Nei's unbiased identities and genetic distances were calculated for all the species of *C. hintonii* complex (Table 4) and clustering using UPGMA (Fig. 3). As expected for pairs of conspecific populations (Crawford 1983), the genetic distance values between all populations were low (Table 4) for the five populations of *C. hintonii*.

When all the four species were taken into account, the range of genetic distance was from 0.003 to 0.325 (Table 4). The *C. hintonii* complex is divided into two clusters: one containing all populations of *C. hintonii*, *C. macvaughii* and *C. melanadenia*, and the second cluster containing only *C. epifanioi*. These two groups separate at the genetic distance of 0.279. Within the first cluster, the population of Tlayahualco (morph I) of *C. hintonii* is more similar genetically to *C. melanadenia*, than to the other species and morphs of *C. hintonii* complex. This situation also occurred with the Infiernillo population (morph III), which is more similar to *C. macvaughii* than the other morphs of *C. hintonii* species (Fig. 3).

Table 3. Nei's genetic diversity (H_S, D_{ST}, H_T, G_{ST}), Wright's F statistics (F_{IS}, F_{IT}, F_{ST}), and an indirect estimate of gene flow (Nem) for five populations of *Caesalpinia hintonii* species at 15 polymorphic loci. *P < 0.05. † Confidence intervals by bootstrap

Locus	H_S	D_{ST}	H_T	G_{ST}	F_{IS}	F_{IT}	F_{ST}	Nem
AcPH-1	0.441	0.040	0.481	0.084	0.000	00.084	0.084	2.726
AcPH-2	0.385	0.237	0.622	0.380	−0.294	0.198	0.380	0.408
APX-1	0.491	0.082	0.573	0.143	0.382	0.470	0.143	1.498
CPX-1	0.387	0.121	0.507	0.238	0.319	0.4481	0.237	0.804
CPX-2	0.130	0.220	0.349	0.629	−0.15	0.624	0.629	0.147
CPX-3	0.096	0.051	0.147	0.348	1.000	1.000	0.348	0.468
EST-1	0.505	0.033	0.538	0.061	−0.359	−0.277	0.060	3.917
EST-2	0.426	0.146	0.572	0.255	−0.275	0.051	0.255	0.730
GOT-2	0.204	0.057	0.261	0.218	0.703*	0.768*	0.218	0.897
LAP-1	0.120	0.006	0.126	0.049	0.535*	0.559*	0.050	4.750
MDH-1	0.497	0.002	0.500	0.005	−0.876	−0.867	0.005	49.75
MNR-1	0.458	0.042	0.500	0.083	0.101	0.176	0.083	2.762
PGI-1	0.327	0.077	0.404	0.192	0.825*	0.859*	0.191*	1.059
PGI-2	0.214	0.066	0.280	0.237	0.774*	0.828*	0.237	0.805
SDH-1	0.197	0.094	0.291	0.323	−0.478	−0.002	0.322	0.526
Mean	0.325	0.085	0.410	0.207	0.031† (−0.220 to 0.295)	0.232† (−0.047 to 0.464)	0.207*† (0.103 to 0.284)	0.958

The mean genetic identity for all pairwise comparisons in the *C. hintonii* complex (I = 0.892) is higher than the identity value reported for congeneric populations (I = 0.670) (Crawford 1983).

Discussion

Hamrick et al. (1979) and Hamrick and Godt (1989) have indicated that geographical distribution is correlated with genetic diversity; consequently, the more restricted taxa tend to be less diverse. Geographically restricted species exhibit significant lower levels of genetic polymorphism than do taxa with widespread distribution (Karron 1987, 1997). In comparison with the mean values given by these authors, estimates of genetic variation (heterozygosity and polymorphism) were higher in the four species of *Caesalpinia*. These values were

Table 4. Matrix of genetic similarities (above diagonal) and mean genetic distances (below diagonal) (Nei 1978) for all studied species and populations of *Caesalpinia hintonii* complex

Population	1	2	3	4	5	6	7	8
1 Valerio Trujano (morph I)	–	0.948	0.979	0.932	0.945	0.952	0.929	0.753
2 Tlayahualco (morph I)	0.053	–	0.948	0.869	0.857	0.867	0.929	0.722
3 Tehuitzingo (morph I)	0.021	0.053	–	0.968	0.979	0.976	0.954	0.808
4 Zicuítaro (morph II)	0.070	0.140	0.032	–	0.976	0.969	0.907	0.744
5 Infiernillo (morph III)	0.056	0.154	0.021	0.242	–	0.997	0.909	0.769
6 *C. macvaughii*	0.049	0.142	0.249	0.031	0.003	–	0.896	0.742
7 *C. melanadenia*	0.073	0.073	0.047	0.076	0.095	0.109	–	0.757
8 *C. epifanioi*	0.283	0.325	0.213	0.262	0.262	0.298	0.278	–

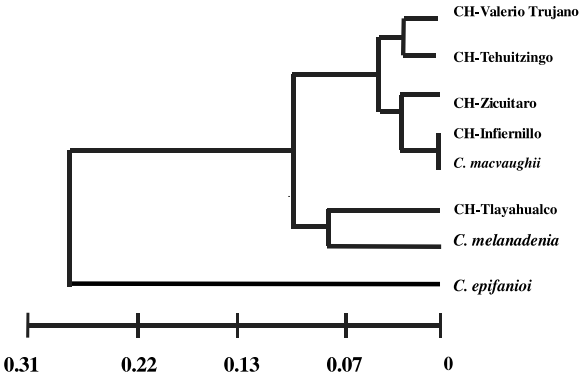

Fig. 3. UPGMA phenogram displaying Nei's genetic distances among the species of the *Caesalpinia hintonii* complex and five populations of *C. hintonii*. CH = *C. hintonii*. Geographic origin of each population is given in Fig. 2

also higher than the average estimated for other legume species (H_o = 0.166, P = 35.96; S. Sotuyo and K. Oyama unpub. data). In addition to this case, several studies on rare and endangered species showed that endemics may actually maintain high levels of genetic variation even within extremely narrow distributions, as was detected in *Delphinium virides-cens* (Richter et al. 1994). These data reinforce previous observations that geographic distribution alone is not a reliable indicator of genetic variability (Karron 1991, Gitzendanner and Soltis 2000).

Historical factors may account for some of the heterogeneity in different levels of genetic polymorphism among restricted or among widespread taxa of a given genus (Hamrick et al. 1981, Gitzendanner and Soltis 2000). The present range of a species may not correspond with its past distribution. Although some restricted taxa have a relatively recent origin and thus, never occupied an extensive range, others might be that were once widespread and have recently declined in their range (Karron 1987). The level of genetic diversity in all taxa may depend on the extent of variation of their ancestral populations and proportions of that variation is represented on founder individuals and thus, do not necessarily reflect the total genetic diversity in this taxon.

The high genetic variation found in most of the populations of the *C. hintonii* complex suggests that they had maintained gene flow until very recent times. This is reinforced by the genetic differentiation value found among these populations and its morphological differentiation in floral traits (Fig. 1). Apparently, the genetic variation of these populations had not been eroded during speciation. However, the most restricted populations with lower genetic variation (morph III of *C. hintonii*, and the single population of *C. epifanioi*) may have gone through bottlenecks after colonization and isolation into their present restricted habitats. Unfortunately, without a more precise historical information and detailed well-supported phylogenies the relative likelihood of these scenarios cannot be distinguished (Godt et al. 1997). Knowledge of the phylogenetic relationships within the *C. hintonii* complex and genetic information regarding it no doubt will provide clues to its history.

The expected heterozygosity under Hardy-Weinberg equilibrium within the *C. hintonii* (H_e = 0.264) populations studied here, was higher than the reported average estimates for endemic species (H_e = 0.163) and for plants with explosive seed dispersal (H_e = 0.217) (Hamrick and Godt 1989). The level of genetic diversity of a plant species has been related with its life form, geographical range, mating system, seed dispersal mechanisms, and with other ecological factors. All studied species of *Caesalpinia* are long-lived perennial plants, and they dispersed their seeds explosively. This combination of traits may be responsible for the high levels of genetic variation observed in the populations of this complex. The apparently high levels of inbreeding (demonstrated by heterozygote deficit (Table 2)) may be explained by a spatial structuring of genetic variation within populations. If gene flow is limited, genetic neighborhoods with different allele frequencies will result in the detection of deficiencies in heterozygotes when entire populations are sampled.

Estimates of allozyme differentiation among populations (F_{ST}) and the proportion of gene diversity residing among populations (G_{ST}) have also been compared among studies of plant species (Hamrick and Godt 1989). Genetic differentiation (F_{ST} = 0.207) among *C. hintonii* populations reflects a considerable differentiation among them. G_{ST} value proved to be lower than the mean average reported for flowering plants (G_{ST} = 0.273), plants with temperate – tropical distribution (G_{ST} = 0.233), or species with explosive seed dispersal (G_{ST} = 0.243) (Hamrick and Godt 1989). Interestingly, endemic species do not, on average, have less diverged populations (G_{ST} = 0.248) compared even with widespread species (G_{ST} = 0.210; Hamrick and Godt 1989). Probably, populations of *C. hintonii* are under genetic divergence as by-product of local adaptations to different habitats, where the genetic structure of these populations is closely in accordance with the spatial distribution of plants. Populations are isolated in all cases, comprising small patches of relatively low density.

Within the species *C. hintonii*, we found that the three distinct forms from Guerrero and Puebla are genetically differentiated, as suggested by Contreras (1991) who described morphological differentiation in the basis of their indumentum, leaflet shape, and distinctive inflorescences and flowers.

The electrophoretic evidence indicates that *C. hintonii* (Infiernillo – morph III) is genetically more similar to *C. macvaughii*. The genetic distance between these two species is almost zero, and it is in accordance with their strong morphological similarity supporting the assumption that these species are closely related. This similarity can be explained by common ancestry, fairly recent divergence. High levels of gene flow between sister taxa or populations may also have contributed to this nested pattern. Furthermore, speciation in peripheral populations (*C. hintonii* – morph III) will result in a pattern where parts of a species are more genetically similar to populations of geographically proximate species than to the genetic conspecifics.

The isolated position in the phenogram of *C. epifanioi* from Ozomatlán, Guerrero is remarkable. Indeed, *C. epifanioi* is known only from a single population with approximately one hundred individuals. It may therefore be questioned whether or not the relatively high differentiation could have been result from recent colonization events. Wade and McCauley (1988) in a theoretical study have shown that founding events may increase differentiation among young populations, depending on the number of individuals involved in the typical founding event and the number of source populations from which they are drawn. On the other hand, high values of genetic distances between species are a reliable indication of a past genetic divergence. This has been shown for several plant taxa, such as the *Helianthus debilis* complex (Wain 1983), the *Lisianthus skinneri* complex (Sytsma and Schaal 1985), Hawaiian *Bidens* (Helenurm and Ganders 1985), and the *Mabrya* complex (Elisens and Crawford 1988).

Morphological (Contreras 1991) and genetical data (this study) indicate that the species of *C. hintonii* complex are discrete, recognizable entities that maintain their genetic integrity although the ranges of some traits may overlap. Thomas (1994) has suggested that the evolutionary success of legumes is due to its "extensive flexible adaptive responses, both in structural and physiological traits". This "developmental plasticity" has influenced the course of evolution by acting as a diversifying factor in the origin of novel traits, and thus, speciation. The genetic distances between species suggest that this youthful group is in a phase of present radiation. Studies on pollination ecology, breeding systems and phylogeography are currently conducted and may help to elucidate the origin of the reproductive isolating mechanisms and thus, the process of speciation involved in the *C. hintonii* complex.

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