Isozyme variation in Calliandra calothyrsus (Leguminosae): its implications for species delimitation and conservation[1]

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ABSTRACT

Patterns of genetic diversity within and among populations of Calliandra calothyrsus Meisn., an important multipurpose tree species, were examined using isozyme analysis. C. calothyrsus is a widespread species distributed throughout Central America and southern Mexico, across a variety of environments. Morphologically and ecologically distinct populations can be identified within this range, but they are currently considered to represent a single species. C. calothyrsus has been introduced to many parts of the tropics, where it is cultivated as a source of fuelwood, animal fodder, green manure, and shade by rural communities. Some of these introductions are known to have originated from Guatemala, but very little is known about the genetic diversity of either the native, or naturalized populations. Isozyme electrophoresis of 23 loci across 17 populations of C. calothyrsus indicated that the majority of genetic diversity was partitioned between populations (F_{ST} = 0.802) and that within-population heterozygosity was low (mean H_o = 0.057). Naturalized populations had lower than expected heterozygosities and were most similar to material from Santa María de Jesus, a natural population in southern Guatemala. Four distinct groups of populations were identified on the basis of Nei's genetic distances and Population Aggregation Analysis (PAA), and correlate with the morphological and ecological differences that can be observed within the species. The results are discussed in relation to species delimitation and conservation.

Key words: Calliandra calothyrsus; isozymes; genetic variation; Leguminosae; population structure; species delimitation.

INTRODUCTION

In recent years, analysis of isozyme variation has been used widely to study genetic diversity within and among populations of neotropical tree species (e.g., Buckley et al., 1988; Hamrick and Murawski, 1991; Hall, Chase, and Bawa, 1994; Chase, Boshier, and Bawa, 1995). Such studies have been generally employed in response to tropical deforestation and its consequences, i.e., the loss of biodiversity and the potential loss of genetic diversity within a species. However, isozyme analysis has also been used to provide information on optimal seed sources for reafforestation when species are introduced and cultivated as exotics. For example, studies of Leucaena Benth. (Schifino-Wittmann and Schlegel, 1990; Harris et al., 1994) and Gliricidia sepium (Jacq.) Walp. (Chalmers et al., 1992; Chamberlain, Galwey and Simons, 1996) provide two cases of the increasing use of fast-growing legume trees that are native to the neotropics, but planted for a range of purposes elsewhere. Such introductions may often be accompanied by loss in genetic diversity if little is known about the original species and its genetic structure. Biodiversity and its potential loss have also renewed interest in taxonomy and species delimitation. The emergence of the phylogenetic species concept (Nixon and Wheeler, 1990) has prompted the increased use of isozyme data in this area of systematics (e.g., Davis and Nixon, 1992; Davis and Goldman, 1993; Elisens and Nelson, 1993; Chamberlain, Hughes, and Galwey, 1996). Fixed differences can be used to provide unique character combinations that can distinguish groups of populations as distinct from one another. The attributes of isozymes, i.e., the identification of individual loci and alleles and the relative ease of assaying many individuals from diverse populations (Gottlieb, 1977), can render them useful in the search for fixed differences among populations.

The present analysis involves Calliandra calothyrsus Meisn. (Leguminosae: Mimosoideae), an early-successional, small leguminous tree that is widely distributed throughout Central America and southern Mexico (Fig. 1; Hernández, 1991; Macqueen, 1992). The species occurs across a considerable range of ecological conditions from very wet (over 5000 mm of rainfall per year) to seasonally dry (as low as 900 mm rainfall per year), and from sea level to altitudes of 1500 m in southern Guatemala. The largest C. calothyrsus trees are found in upland areas of moderate to high rainfall where they can reach 10 m in height, are usually single stemmed and characterized by the possession of a dark, reddish-brown bark and flowers with bright red staminal filaments. In drier regions, trees are generally small (to 5 m in height), single or multiple stemmed with pale grey bark and flowers with staminal filaments that are white at the base and pink at the tips. C. calothyrsus is a riverine colonizer in natural undisturbed forest, but is now a very common element of secondary vegetation in areas of disturbance such as roadsides and fallow agricultural land. It often appears in thickets during early colonization, but with time tends to be outcompeted and has a scattered distribution in older stands, sometimes over several kilometres. Disturbance, both natural and as a result of human activity, is a frequent occurrence in the majority of environments where C. calothyrsus grows. Natural disturbance may occur via changes in the level or course of a river, whereas human disturbance may result from populations in secondary vegetation being cut or burnt. C. calothyrsus is a precocious flowerer with an abundant and prolonged nightime floral display. The species is bat-pollinated and outcrossing (Hernández, 1991; Macqueen, 1992), but tolerates selfing at a low level (Chamberlain, in press). Seeds are dispersed from the pods by explosive apical dehiscence, releasing seed over a range of 0-10 m (Macqueen, 1992).

Calliandra calothyrsus has been cultivated and used widely for the provision of fuelwood, animal fodder, green manure, shade and soil conservation in many parts of the humid tropics (National Research Council, 1983; Macqueen, 1992, 1993). The species was introduced to Indonesia from Guatemala in the 1930s (Verhoef, 1939) and is now naturalized in many parts of Java. Seed from these naturalized populations has been the major source of material for subsequent planting in south east Asia, Australia, and Africa, without much thought to, or knowledge of, its origin and genetic diversity. To remedy this situation, a research program was initiated in 1990 at the Oxford Forestry Institute (OFI) to map the natural distribution of C. calothyrsus and collect seed for an

international network of provenance trials (Macqueen, 1993). Under this program, seed was collected from 48 populations throughout the natural geographic distribution of the species, covering a range of climatic and altitudinal zones in Central America and Mexico.

Despite recent taxonomic investigations, the status and relationships of C. calothyrsus remain confused. The species has been known under a variety of synonyms, including Calliandra confusa Sprague & Riley, Calliandra similis Sprague & Riley and Calliandra acapulcensis Britton & Rose (Standley) (Macqueen and Hernández, 1997). Woodson and Schery (1950) believed C. confusa and C. similis to be extremes of the same taxonomic entity, and officially sank the two into synonymy. Breteler (1989) officially sank C. confusa into synonymy with C. calothyrsus, and more recently Macqueen and Hernández (1997) have treated all of the above species as conspecific with C. calothyrsus. The latter authors consider C. calothyrsus to be a member of the series Racemosae, a group of seven closely related species, whereas R. Barneby (unpublished data, New York Botanical Garden), in his revision of the whole of the genus Calliandra, subsumes the Racemosae within series Calliandra and treats C. calothyrsus as a variety of Calliandra houstoniana (Mill.) Standley along with a number of other previously separate species.

The objective of the current study was to explore patterns of genetic variation in C. calothyrsus. The survey was based on the isozyme analysis of populations selected from both the species' natural range and from where it is cultivated. The results are interpreted in the context of species delimitation, the likely origins of naturalized populations and conservation of the genetic resources of this important legume species.

MATERIALS AND METHODS

Plant material -- Fifteen populations of C. calothyrsus, representative of its natural distribution, and two naturalized populations were sampled from the OFI seed collection (Table 1; Fig. 1). Populations were sampled in two ways: (1) seeds from a large number of individual trees within a population were bulked (Zihuatanejo, Copán, Meambar, Santa María, El Volcán, Maduin, and Embu) and 60 randomly sampled seeds examined from the bulk collection; (2) seeds from between 11 and 25 families (individual trees) per population (Bombana, Barillas, Cobán, Santa María de Jesus, Patulul, La Ceiba, San Ramón, La Puerta, Fortuna, and Turrialba) were collected in the field. Each family comprised seed progeny from a single maternal parent, and a minimum distance of 100 m separated each family to avoid bias due to co-ancestry (Macqueen, 1992). Between five and seven seeds per family were randomly sampled for examination.

Enzyme extraction and electrophoresis -- Isozyme electrophoresis was carried out on 12% starch using three buffer systems: (1) Histidine-citrate pH 5.7 - electrode buffer, which comprised 10.09 g L-histidine per litre distilled water (pH 5.7 with citric acid), and gel buffer, which comprised 1:6 dilution of electrode buffer; (2) Tris-citrate pH 7.5 (Chamberlain, Hughes, and Galwey, 1996); and (3) Lithium-borate pH 8.3 (Chamberlain, Hughes, and Galwey, 1996). Half of one seed cotyledon, left soaking in water overnight, was ground in three drops of extraction buffer comprising 50 mL lithium-borate gel buffer, 37 mg potassium chloride, 10 mg magnesium chloride, 18 mg EDTA (tetrasodium), 25 mg PVPP, 0.5 mL Triton-X-100, and 2 mL ß-mercaptoethanol. Enzyme extracts were absorbed onto filter paper wicks, loaded onto the starch gels, and run at 4^{o}C until the 1% bromophenol blue tracker dye had migrated 8 cm from the origin. Multi-population samples were used to standardize the interpretation of results. After a preliminary survey to identify potentially polymorphic isozymes, ten enzyme systems were used to resolve isozymes: aconitase (ACO - E.C. 4.2.1.3; buffer system 2), alcohol dehydrogenase (ADH - E.C. 1.1.1.1; buffer system 3), esterase (EST - E.C. 3.1.1.-; buffer system 3), isocitrate dehydrogenase (IDH - E.C. 1.1.1.42; buffer system 2), malate dehydrogenase (MDH - 1.1.1.37; buffer system 1), 6-phosphogluconate dehydrogenase (6-PGD - E.C. 1.1.1.44;

buffer system 2), phosphoglucose isomerase (PGI - E.C. 5.3.1.9; buffer system 3), phosphoglucomutase (PGM - E.C. 2.7.5.1; buffer system 1), shikimate dehydrogenase (SDH - E.C. 1.1.1.25; buffer system 2) and superoxide dismutase (SOD - E.C. 1.15.1.1; buffer system 3).

Analysis of isozyme polymorphisms -- As full-sib progeny were not available, and as the sampled trees were diploid and open-pollinated (Macqueen, 1993), genetic interpretations of the isozyme gel banding patterns were based on the evaluation of isozyme polymorphisms in other well-documented investigations (Wendel and Weeden, 1989). Such interpretations have been corroborated by genetic analysis, and the subunit structure of many isozymes is known (Shields, Orton, and Stuber, 1983). In most cases, the banding pattern implied a simple diploid genetic model and could be interpreted in terms of loci and alleles. A genotype was assigned to each seed on this basis, and the allele frequencies at each locus in each population were calculated (summarized in Table 2) and entered into BIOSYS-1 (Swofford, 1989). Measures of heterozygosity within and between populations were computed. Nei's genetic distances (Nei, 1978) between all pairs of populations were computed and used to cluster the populations by the unweighted pair group method with arithmetic averaging (UPGMA). Species delimitation was investigated using Population Aggregation Analysis (PAA; Davis and Nixon, 1992; Davis and Goldman, 1993), a method that enables the identification of phylogenetic species (Nixon and Wheeler, 1990). Briefly, this procedure begins by scoring each allele (x) in each population as either absent (0), present and fixed (1), or present and not fixed (*; $0 < x < 1$). Each population allele profile is then compared with all others, and the populations are 'aggregated' when they are not distinct from one another, i.e., when they do not differ by the fixed occurrence of at least one allele in one population and its absence from the other. In this way, multi-population species, characterized by the occurrence of a unique fixed character combination, are identified, meeting the requirement of the phylogenetic species concept that descent relationships among such populations are hierarchic. PAA was conducted manually.

RESULTS

Isozyme variation -- Five of the ten enzyme systems analyzed were interpreted as monomers (ACO, EST, MDH, SDH, PGM), and the remaining five as dimers (ADH, IDH, 6-PGD, PGI, SOD), two of which had overlapping loci, i.e. ADH and 6-PGD. All the enzyme systems analyzed were polymorphic either within- or between-populations (see Table 2). The MDH loci and 6-Pgd-3 locus exhibited no within-population variation and had fixed monomorphic alleles in each population. All other loci were polymorphic within at least one population.

Genetic diversity within populations -- The percentage polymorphic loci with any polymorphism detected ranged from 0.00 to 34.78% with a mean of 20.46% (Table 3). All enzyme systems were polymorphic for two or more alleles. The average number of alleles per locus ranged from 1.04 to 1.61 with a mean of 1.22. The average genetic diversity (H_o) based on 23 loci was 0.057 for all the populations surveyed. The observed heterozygosity values ranged from 0.001 to 0.115. When the data across all populations within each group defined by Nei's (1978) unbiased genetic distance (see Fig. 2) was pooled, the most diverse group was that containing the population from Zihuatanejo, Mexico, and the least diverse was Group II containing populations from Costa Rica and Panama. There were significant deviations from Hardy-Weinberg expectations ($P<0.05$) for the populations at Cobán, Patulul, Maduin, and Embu.

Genetic diversity between populations -- F_{ST} compares the ratio of the between population component of diversity to the total diversity. In this survey, F_{ST} for all populations was estimated as 0.802, which indicates that the between-population component accounts for approximately 80% of the detected variation (Table 4).

Genetic distance -- A dendrogram based on Nei's (1978) unbiased genetic distances is shown in Fig. 2. Based on individual pairwise comparisons, the mean genetic distance (D) was 0.330 with a range of 0.000 to 0.577. The dendrogram defines four main clusters: Group I is composed of nine populations from Mexico, Guatemala, northern Honduras and the naturalized populations, Maduin and Embu; Group II is composed of three populations from Costa Rica and Panama; Group III is composed of five populations from the inland, upland areas of Honduras and Nicaragua; and Group IV is composed of the population from Zihuatanejo, Mexico. F - statistics were also calculated for the population groups defined by Nei's genetic distances (Table 4). A F_{ST} value of 0.086 was obtained for the Group III, but the F_{ST} values for Groups I and II were much higher (F_{ST} = 0.469 and F_{ST} = 0.327 respectively.

Genetic diversity and rainfall patterns -- The correlations between rainfall, altitude, latitude, and the number of fixed alleles per population were examined. A pattern of increasing number of fixed alleles is observed with increasing mean annual rainfall (Fig. 3). Regression analysis indicates that 63% (F = 22.36, P = 0.00) of the variability observed can be explained by this relationship. A plot of the mean observed heterozygosity vs. rainfall (graph not shown) shows a corresponding decrease with increasing rainfall (r^2 = 0.39; F = 8.35, P = 0.013).

Population aggregation analysis (PAA) -- PAA of all sampled populations of C. calothyrsus aggregates them into four isozyme taxa that correspond to the clusters defined by Nei's genetic distances (Table 5). The nine populations from Mexico, Guatemala, the north coast of Honduras, and the naturalized populations (isozyme taxon C. calothyrsus-1; genetic distance Group I) are distinct from all other populations on the basis of the fixed occurrence of Mdh-1b. The three populations from Costa Rica and Panama (isozyme taxon C. calothyrsus-2; genetic distance Group II) are distinct from all other populations on the basis of the fixed occurrence of 6-Pgd-3b. The five populations from the inland, upland areas of Honduras and Nicaragua (isozyme taxon C. calothyrsus-3; genetic distance Group III) are distinct from all other populations on the basis of the fixed occurrence of Mdh-4b. The population from Zihuatanejo (isozyme taxon C. calothyrsus-4; genetic distance Group IV) shares alleles in common with the populations from Honduras and Nicaragua, but is different from all other populations on the basis of the fixed occurrence of Mdh-1c.

DISCUSSION

Species delimitation -- Isozyme data allow quantification of the similarity, or difference, between populations, groups of populations and species (Gottlieb, 1977, 1981). Populations and/or species can be characterized on the basis of differences in allele frequencies. What has been recognized for some years now, and has perhaps meant that enzyme electrophoresis has not been routinely used by plant taxonomists, is that divergence of genes specifying soluble enzymes is often uncorrelated with plant speciation (Gottlieb, 1973, 1974; Gottlieb and Pilz, 1976). Population aggregation analysis, however, specifically sets out to utilize isozyme data for the delimitation of phylogenetic species sensu Nixon and Wheeler (1990), and has been used for this purpose by Davis and Goldman (1993), Elisens and Nelson (1993), Vogler and DeSalle (1994), and Chamberlain, Hughes, and Galwey (1996). Using PAA, populations and/or species can be characterized on the basis of the occurrence of fixed alleles, as opposed to the frequency of those alleles. Among the populations of C. calothyrsus studied, the strict standards imposed by PAA reveal four distinct isozyme taxa (C. calothyrsus-1 through -4). The cluster analysis of Nei's genetic distances and the high value of F_{ST} (0.802) also provide evidence for the existence of distinct groups within the species. These population aggregates correspond closely to broad geographical, ecological, and morphological boundaries. The populations of C. calothyrsus-1 occur in areas of Mexico, Guatemala, and Honduras with moderate to high rainfall. On the Pacific slopes, trees generally occur at altitudes of between 1000 and 1500 m, whereas on the Caribbean coastal plains and hills, the species is found

close to sea level. Trees are generally taller (up to 10 m in height), usually single stemmed (the exception being trees from the population at La Ceiba, Honduras, which are generally multiple stemmed) and possess dark, reddish-brown bark and flowers with bright red staminal filaments. The populations of C. calothyrsus-2 occur in Costa Rica and Panama at mid elevations, and generally experience high annual rainfall. The trees are either single or multiple stemmed, reach average heights of 4-5 m, but taller specimens can be found, have mid-brown bark and flowers with bright red to pink staminal filaments. Trees from this locality were originally described as C. similis (Sprague and Riley, 1923), but were later treated as conspecific with C. calothyrsus by Hernández (1991). The populations of C. calothyrsus-3 are found inland, in the driest regions of the distribution and at mid elevations. Trees are generally small (up to 5 m in height), single or multiple stemmed and possess pale grey bark, larger leaflets and flowers with staminal filaments that are white at the base and pink at the tips. The population from Zihuatanejo (C. calothyrsus-4) is found in the seasonally dry coastal zone of Guerrero, Mexico. Trees from this area share many morphological similarities with those of C. calothyrsus-3, and correspond to what had been previously described as C. acapulcensis (Macqueen and Hernández, 1997). Based on canonical variance analysis (CVA) of quantitative leaf and pod traits, Macqueen (1993) treated C. acapulcensis as conspecific with C. calothyrsus. However, the C. calothyrsus populations measured and used in the CVA analysis were either from the dry areas of Honduras and Nicaragua, or from Costa Rica, and did not include material from the entire range of the species. These two population groups were shown to be morphologically distinct, with C. acapulcensis showing a strong affinity to the Honduran/Nicaraguan populations.

In their revision of Britton and Rose's series Racemosae, Macqueen and Hernández (1997) recognize C. calothyrsus and six closely related species: C. houstoniana, Calliandra grandiflora (L'Her.) Benth., Calliandra juzepczukii Standl., Calliandra longpedicellata (McVaugh) Macqueen & H.M. Hern., Calliandra palmeri S. Watson and Calliandra physocalyx H.M. Hern. & M. Sousa. In contrast, in his revision of the entire genus, R. Barneby (unpublished data, New York Botanical Garden) treats C. calothyrsus as a variety of C. houstoniana along with C. grandiflora and C. acapulcensis. These contrasting species delimitations appear to reflect the wide focus of Barneby's revision of a genus that he recognises to comprise 132 species, and the greater depth of analysis that Macqueen and Hernández have been able to apply to a small section within it. In addition to the seven species Macqueen and Hernández recognize within the series Racemosae, they have also delimited three subspecies of C. houstoniana (all previously ascribed to C. houstoniana) on the basis of a number of floral, pinnae, and pod characteristics. The evidence from PAA of isozyme variation presented here supports the recognition of four segregate taxa within C. calothyrsus more in line with Macqueen and Hernández's approach to species delimitation, than that of Barneby's. However, given the current confusion, formal recognition of these aggregates would be premature until both a morphological analysis of the isozyme taxa delimited in C. calothyrsus is carried out, and the isozyme similarities of C. houstoniana with C. calothyrsus can be assessed.

Genetic diversity -- C. calothyrsus is an increasingly important component of agroforestry systems in many parts of the humid tropics. Considerable efforts are being made to evaluate the genetic resources of it and its close relatives in field trials (Macqueen, 1993; Pottinger, 1996). There has, however, been no attempt to assess levels and patterns of genetic diversity within the species. Levels of isozyme variation found within populations of C. calothyrsus (mean H_o = 0.057) are lower than the average value for animal-pollinated (0.167), early-successional (0.109) or widespread (0.204) tropical tree species (Loveless and Hamrick, 1984; Hamrick and Godt, 1989; Loveless, 1992). Cross-species comparisons can be difficult, and it is possible that estimates of genetic diversity within C. calothyrsus, although typically done, are biased by the inclusion of loci that were polymorphic between, but not within, populations. Alternatively, the relatively low levels of within-population diversity in C. calothyrsus could more likely be attributed to selection pressures experienced by populations in their natural habitats. C. calothyrsus is early successional and appears to thrive in disturbed environments. Human disturbance, however, may have a greater effect on population

diversity than disturbance occurring under natural conditions. In areas of secondary vegetation, trees often occur in small scattered stands, which may be frequently cut or burnt by local people, hence removing a proportion of the reproductive population. This could result in the loss of alleles, either temporarily within one reproductive cycle, or permanently, depending on the extent of the damage. For example, in 1995, the population at Copán, Honduras was almost entirely destroyed due to the upgrading and widening of a nearby road, leaving only a few scattered individuals and a much reduced genepool. Such an effect may be compounded by the ability of the species to tolerate selfing, despite possessing a gametophytic self-incompatibility system (Chamberlain, in press), and inbreeding will result. The lowering of genetic diversity as a result of human activity could have a widespread effect given that the majority of natural C. calothyrsus populations have spread from rivers within disturbed forest into neighbouring pasture or roadsides.

It is interesting that heterozygosity in C. calothyrsus was found to be inversely proportional to rainfall, a relationship found in another neotropical tree species, Cordia alliodora (R. and P.) Oken (Chase, Boshier, and Bawa, 1995). One argument used to explain this correlation in C. alliodora is that habitat diversity, and hence genetic diversity within plant populations, may be greater in the dry than in the wet zone due to the selection pressures imposed by water availability. The same could also be true of C. calothyrsus, although there also may be a number of other factors external to the relationship observed. For example, trees that comprise the population at Fortuna, Costa Rica (H_o = 0.009; rainfall = 4718 mm/yr) are scattered over approximately 12 km in a river valley and nearby roadsides. Flowering is highly asynchronous between stands of C. calothyrsus in this area, hence gene flow between stands will be limited, genetic bottlenecks may have occurred, and selfing and/or consanguineous mating within stands may have been promoted. Trees that comprise the population at Bombana (H_o = 0.008; rainfall = 1256 mm/yr) occur in a river valley in a seasonally dry zone of Mexico. The population is confined to the humid valley floor and is isolated from other sources of C. calothyrsus by the surrounding arid vegetation. Gene flow will therefore have been restricted, genetic bottlenecks may have occurred, and selfing and/or consanguineous mating promoted.

C. calothyrsus is known to have been introduced from Guatemala to Indonesia by Dutch botanists in 1936 (Verhoef, 1939). It was introduced as coffee shade, along with a number of other legume species, as a substitute for Leucaena leucocephala (Lam.) de Wit.. Isozyme variation shows that the Indonesian and Kenyan populations of C. calothyrsus are most similar to the populations from Mexico and Guatemala, particularly the population from Santa María de Jesus, Guatemala. Both populations, however, exhibit allele c at the Pgm-1 locus, which is not found in the native population at Santa María de Jesus. This allele is found in the nearby population at Patulul, suggesting that the original introductions may have been made from more than one population in this area of southern Guatemala. Both the Indonesian and Kenyan populations have lower than expected heterozygosities and are in Hardy-Weinberg disequilibrium. This may also suggest a multi-population origin of these stands, or that the native populations were not sampled for seed evenly, resulting in loss of genetic diversity. It can be noted, however, that the expected heterozygosities and the mean number of alleles per locus for the naturalized populations are amongst the highest across all 17 populations sampled. It is possible that allelic variation has been retained in the naturalized populations, which often form large undisturbed stands, whereas the native populations have been more frequently disturbed with successive genetic bottlenecks causing the loss of allelic variation over time.

Conservation status -- Given the widespread distribution of C. calothyrsus throughout Mesoamerica, conservation at the species level is not a problem. At the population level, however, the species may be under threat of degradation. Populations of C. calothyrsus may have been exposed to genetic bottlenecks now and in the past as a result of disturbance and recolonization. The species does, however, appear to thrive in these situations, but combined with the effect of inbreeding, the result may be unpredictable in terms of within-species diversity. Where disturbance occurs in natural forests, i.e., within a river system, genetic bottlenecks may have a relatively mild effect, and the

population could recover its genetic equilibrium in a few generations. Where disturbance has occurred under the influence of human activity, however, the effect on the population may be much more severe and the results unpredictable in terms of the recovery of genetic diversity. It is clear, however, that the high level of between-population diversity within the species must be used to prioritize populations for conservation. Groups of populations characterized by fixed isozyme alleles defines their members as distinct in terms of the phylogenetic species concept. Some systematists (e.g., Cracraft, 1991; Rojas, 1992) consider 'phylogenetic species' to be equivalent to 'evolutionarily significant units' (ESU; Ryder, 1986), a concept employed by conservationists that is central to the evolutionary history of populations and hence, the evaluation of their conservation status. Vogler and DeSalle (1994), however, 'propose that ESUs should not be equated specifically with the term 'species'. Rather it should be used as a technical term for clusters of organisms that are evolutionarily distinct and hence merit separate protection.' Whatever the definition of terminology used, there is evidence that the populations comprising each of the isozyme taxa C. calothyrsus-1 to -4 are evolutionarily distinct and as such should be given special attention when considering conservation of the species as a whole.

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Table 1. Locations of the 17 Calliandra calothyrsus populations analyzed and the number of families sampled for isozyme analysis. Figures in parentheses are the number of individuals sampled per family or bulk seedlot. All vouchers are >D.J. Macqueen= unless otherwise stated and are deposited at K, MEXU and FHO.

Population	**OFI No.**	**Voucher**	**Lat (N)**	**Long (W)**	**Mean rainfall (mm/yr)**	**No. families**
Bombana, Mexico	31/93	315	16°56'	93°02'	1256	20 (5)
Zihuatanejo, Mexico	64/92	171	17°40'	101°30'	1102	bulk (60)
Barillas, Guatemala	35/93	358	15°46'	91°19'	5829	14 (5)
Cob<n, Guatemala	8/91	60	15°28'	90°15'	2517	20 (5)
Santa MarRa de Jesus, Guatemala	33/93	12	14°45'	91°32'	4236	15 (5)
Patulul, Guatemala	9/91	13	14°24'	91°09'	3185	16 (5)
La Ceiba, Honduras	15/95	29	15°43'	86°50'	2884	18 (6)
Copan, Honduras	23/91	22	14°50'	89°08'	1688	bulk (60)
Meambar, Honduras	23/84	1	14°58'	87°46'	2218	bulk (60)
Santa MarRa, Honduras	13/91	98	14°07'	86°12'	1145	bulk (60)
San Ram\n, Nicaragua	110/94	3	12°54'	85°48'	1394	13 (6)
La Puerta, Nicaragua	109/94	6[a]	12°11'	85°15'	1889	11 (7)
Fortuna, Costa Rica	108/94	115	10°30'	84°48'	4718	12 (7)
Turrialba, Costa Rica	14/95	99	9°55'	83°42'	2363	25 (5)
El Volc<n, Panama	61/93	597	8°45'	82°24'	3517	bulk (60)
Maduin, Indonesia	147/91	123	7°36'S	111°30'E	1884	bulk (60)
Embu, Kenya	13/95	[b]	0°30'S	37°21'E	1252	bulk (60)

[a] D.J. Macqueen and B.T. Styles
[b] No voucher collected

Table 2. Summary of allele frequencies for 23 isozyme loci among all populations surveyed.

Locus	Allele	Population																
		Zihuatanejo	**Bombana**	**Barillas**	**Cob<n**	**Santa MarRa de Jesus**	**Patulul**	**La Ceiba**	**Cop<n**	**Meambar**	**Santa MarRa**	**San Ram\n**	**La Puerta**	**Fortuna**	**Turrialba**	**El Volc<n**	**Maduin**	**Embu**
Aco-1	a	0.477	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.438	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.960	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.085	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Aco-2	a	0.000	1.000	1.000	0.000	0.352	0.079	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.438	0.087
	b	0.000	0.000	0.000	0.137	0.401	0.902	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.073	0.000	0.450	0.913
	c	0.015	0.000	0.000	0.863	0.247	0.018	0.000	0.000	0.000	0.070	0.052	0.006	1.000	0.927	1.000	0.112	0.000
	d	0.985	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.930	0.948	0.994	0.000	0.000	0.000	0.000	0.000
Adh-1	a	1.000	0.981	1.000	0.892	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.862	1.000	1.000	1.000
	b	0.000	0.019	0.000	0.108	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138	0.000	0.000	0.000
Adh-2	a	0.815	0.990	1.000	1.000	1.000	0.012	1.000	0.025	0.000	0.000	0.012	0.000	1.000	1.000	1.000	0.975	1.000
	b	0.185	0.010	0.000	0.000	0.000	0.988	0.000	0.975	1.000	1.000	0.988	1.000	0.000	0.000	0.000	0.025	0.000
Est-1	a	1.000	0.967	1.000	0.142	1.000	0.787	0.873	0.975	0.613	0.910	0.884	0.819	1.000	0.595	1.000	0.475	0.800
	b	0.000	0.033	0.000	0.858	0.000	0.213	0.127	0.025	0.375	0.090	0.116	0.144	0.000	0.405	0.000	0.525	0.200
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000
Est-2	a	0.000	0.090	0.000	0.789	0.000	0.000	0.534	0.000	0.000	0.000	0.000	0.000	0.000	0.625	0.000	0.225	0.350
	b	0.000	0.910	1.000	0.211	1.000	1.000	0.466	0.000	0.000	0.000	0.000	0.000	1.000	0.375	1.000	0.775	0.650

Locus	Allele	Population																
		Zihuatanejo	**Bombana**	**Barillas**	**Cob<n**	**Santa MarRa de Jesus**	**Patulul**	**La Ceiba**	**Cop<n**	**Meambar**	**Santa MarRa**	**San Ram\n**	**La Puerta**	**Fortuna**	**Turrialba**	**El Volc<n**	**Maduin**	**Embu**
	c	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Est-3	a	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.237	0.625	0.560	0.500	0.356	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.762	0.375	0.440	0.500	0.644	0.000	0.000	0.000	0.000	0.000
	c	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Idh-1	a	0.885	1.000	1.000	0.912	0.852	0.841	1.000	1.000	1.000	0.970	1.000	0.981	1.000	1.000	1.000	0.913	1.000
	b	0.115	0.000	0.000	0.088	0.148	0.159	0.000	0.000	0.000	0.030	0.000	0.019	0.000	0.000	0.000	0.087	0.000
Mdh-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
	b	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	c	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	a	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	a	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Mdh-4	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Mdh-5	a	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	b	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000

Locus	Allele	Population																
		Zihuatanejo	Bombana	Barillas	Cob<n	Santa MarRa de Jesus	Patulul	La Ceiba	Cop<n	Meambar	Santa MarRa	San Ram\n	La Puerta	Fortuna	Turrialba	El Volc<n	Maduin	Embu
6-Pgd-1	a	0.185	1.000	1.000	1.000	1.000	1.000	1.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	b	0.815	0.000	0.000	0.000	0.000	0.000	0.000	0.875	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
6-Pgd-2	a	0.423	0.000	0.000	0.000	0.000	0.000	0.0001.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000
	b	0.577	1.000	1.000	1.000	1.000	1.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
6-Pgd-3	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
Pgi-2	a	0.015	0.000	0.000	0.000	0.000	0.049	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000
	b	0.985	1.000	1.000	1.000	1.000	0.951	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000
Pgm-1	a	0.031	0.000	0.000	0.059	0.407	0.195	0.000	0.500	0.575	0.420	0.448	0.356	0.171	0.328	0.000	0.200	0.275
	b	0.062	1.000	1.000	0.941	0.593	0.537	1.000	0.213	0.287	0.000	0.116	0.356	0.000	0.000	0.000	0.675	0.438
	c	0.908	0.000	0.000	0.000	0.000	0.268	0.000	0.287	0.138	0.580	0.436	0.287	0.829	0.672	1.000	0.125	0.287
Pgm-2	a	0.238	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.162	0.090	0.047	0.100	0.000	0.000	0.000	0.000	0.175
	b	0.000	0.000	0.007	0.358	0.093	0.201	0.475	0.000	0.000	0.200	0.000	0.000	0.000	0.456	0.071	0.000	0.075
	c	0.762	1.000	0.993	0.642	0.852	0.799	0.525	1.000	0.837	0.710	0.953	0.900	1.000	0.544	0.929	1.000	0.750
Pgm-3	a	1.000	0.990	1.000	0.000	0.994	1.000	0.945	0.863	0.712	0.780	0.965	0.950	0.000	0.000	0.000	1.000	0.837
	b	0.000	0.010	0.000	0.000	0.006	0.000	0.055	0.138	0.287	0.220	0.035	0.050	0.000	0.000	0.000	0.000	0.162
	c	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000

Locus	Allele	Population																
		Zihuatanejo	**Bombana**	**Barillas**	**Cob<n**	**Santa MarRa de Jesus**	**Patulul**	**La Ceiba**	**Cop<n**	**Meambar**	**Santa MarRa**	**San Ram\n**	**La Puerta**	**Fortuna**	**Turrialba**	**El Volc<n**	**Maduin**	**Embu**
Sdh-1	a	0.977	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sdh-2	a	0.108	0.000	0.000	0.000	0.000	0.000	0.000	0.563	0.213	0.350	0.360	0.688	0.000	0.000	0.000	0.000	0.000
	b	0.885	0.000	0.000	0.000	0.000	0.000	0.000	0.425	0.788	0.640	0.640	0.313	0.000	0.000	0.000	0.000	0.000
	c	0.008	1.000	1.000	1.000	1.000	1.000	1.000	0.013	0.000	0.010	0.000	0.000	1.000	1.000	0.946	1.000	1.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000
Sod-2	a	0.000	1.000	1.000	1.000	1.000	1.000	0.640	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	1.000	0.000	0.000	0.000	0.000	0.000	0.360	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 3. Genetic diversity estimates for all populations surveyed. Statistics are based on 17 polymorphic loci and six monomorphic loci calculated for all enzyme systems (standard errors in parentheses). Percentage polymorphic loci (P) are calculated as the number of loci with the most common allele frequency <0.95 divided by the total number of loci. The average number of alleles (A) is the number of alleles >0.05 per locus divided by the number of loci. The observed (H_o) and expected (H_e) heterozygosities are calculated as the average for each locus.

Population	**P**	**A**	**H_o**	**H_e[a]**
Group I				
Bombana	4.35	1.22 (0.09)	0.008 (0.006)	0.013 (0.008)
Barillas	0.00	1.04 (0.06)	0.001 (0.001)	0.001 (0.001)
Cob<n	30.43	1.30 (0.10)	0.046 (0.022)*	0.076 (0.028)
Santa MarRa de Jesus	17.39	1.30 (0.13)	0.068 (0.035)	0.073 (0.037)
Patulul	21.74	1.39 (0.14)	0.051 (0.022)*	0.080 (0.033)
La Ceiba	21.74	1.22 (0.09)	0.064 (0.030)	0.078 (0.035)
Maduin	26.09	1.39 (0.14)	0.051 (0.024)*	0.098 (0.040)
Embu	26.09	1.35 (0.13)	0.047 (0.020)*	0.099 (0.040)
Mean	**18.48**	**1.28 (0.11)**	**0.042 (0.020)**	**0.065 (0.028)**
Group II				
Fortuna	4.35	1.09 (0.06)	0.009 (0.007)	0.014 (0.012)
Turrialba	26.09	1.26 (0.09)	0.087 (0.046)	0.117 (0.050)
El Volc<n	8.70	1.09 (0.06)	0.008 (0.006)	0.010 (0.007)
Mean	**13.05**	**1.15 (0.07)**	**0.035 (0.020)**	**0.047 (0.023)**
Group III				
Cop<n	21.74	1.39 (0.14)	0.066 (0.028)	0.090 (0.038)
Meambar	26.09	1.35 (0.13)	0.095 (0.035)	0.112 (0.042)
Santa MarRa	30.43	1.48 (0.14)	0.105 (0.036)	0.117 (0.039)
San Ram\n	21.74	1.39 (0.12)	0.077 (0.032)	0.089 (0.037)
La Puerta	26.09	1.43 (0.14)	0.077 (0.031)	0.096 (0.039)
Mean	**25.22**	**1.41 (0.13)**	**0.084 (0.032)**	**0.101 (0.039)**
Group IV				
Zihuatanejo	34.78	1.61 (0.15)	0.115 (0.041)	0.119 (0.037)
Overall mean	**20.46**	**1.22 (0.10)**	**0.057 (0.024)**	**0.075 (0.037)**

[a] Nei=s (1978) unbiased estimate

* Significant deviation from Hardy-Weinberg expectations at P<0.05

Table 4. Summary of F statistics calculated within and among the four population groups defined by Nei=s unbiased genetic distances.

Population group	Locus	F_{IS}	F_{IT}	F_{ST}
Group I	Aco-2	0.234	0.715	0.628
	Adh-1	1.000	1.000	0.881
	Adh-2	1.000	1.000	0.949
	Est-1	0.534	0.735	0.430
	Est-2	0.392	0.634	0.398
	Idh-1	0.127	0.192	0.074
	Pgi-2	0.610	0.625	0.038
	Pgm-1	0.328	0.477	0.222
	Pgm-2	0.166	0.328	0.194
	Pgm-3	0.674	0.707	0.101
	Sod-2	0.320	0.544	0.330
	Mean	**0.346**	**0.653**	**0.469**
Group II	Aco-2	0.175	0.216	0.050
	Adh-1	0.469	0.480	0.021
	Adh-2	1.000	1.000	0.096
	Est-1	0.356	0.557	0.312
	Est-2	0.320	0.678	0.526
	Pgm-1	0.368	0.449	0.129
	Pgm-2	-0.116	0.410	0.471
	Mean	**0.328**	**0.548**	**0.327**
Group III	Aco-1	-0.042	-0.008	0.032
	Aco-2	0.267	0.293	0.035
	Adh-2	1.000	1.000	0.014
	Est-1	-0.024	0.088	0.110
	Est-3	0.174	0.240	0.080
	Idh-1	0.237	0.249	0.016
	Pgm-1	0.277	0.328	0.070
	Pgm-2	0.294	0.358	0.090
	Pgm-3	0.028	0.102	0.076
	6-Pgd-1	0.086	0.179	0.103
	Sdh-2	0.053	0.163	0.115
	Mean	**0.157**	**0.229**	**0.086**
All populations	Aco-1	-0.046	0.417	0.442
	Aco-2	0.230	0.863	0.821
	Adh-1	1.000	1.000	0.800
	Adh-2	1.000	1.000	0.941
	Est-1	0.309	0.548	0.346
	Est-2	0.377	0.863	0.781
	Est-3	0.174	0.786	0.741
	Idh-1	0.090	0.167	0.084
	Mdh-1	-	1.000	1.000
	Mdh-2	-	1.000	1.000
	Mdh-3	-	1.000	1.000
	Mdh-4	-	1.000	1.000
	Mdh-5	-	1.000	1.000
	Pgi-2	0.523	0.542	0.038
	Pgm-1	0.300	0.608	0.440

Pgm-2	0.128	0.329	0.230
Pgm-3	0.197	0.805	0.757
6-Pgd-1	0.023	0.940	0.939
6-Pgd-2	-0.292	0.926	0.943
6-Pgd-3	-	1.000	1.000
Sdh-1	-0.024	-0.001	0.022
Sdh-2	0.092	0.739	0.712
Sod-2	0.320	0.875	0.816
Mean	**0.238**	**0.850**	**0.802**

Table 5. Population Aggregation Analysis (PAA) for 17 populations of Calliandra calothyrsus using 23 isozyme loci (* allele present, not fixed; 1 allele present, fixed; 0 allele absent).

Population	**Locus**																						
	ACO		ADH		EST			IDH	MDH					6-PGD			PGI	PGM			SDH		SOD
	1	2	1	2	1	2	3	1	1	2	3	4	5	1	2	3	2	1	2	3	1	2	2
	a b c	a b c d	a b	a b	a b c	a b c	a b c	a b	a b c	a b	a b	a b	a b	a b	a b	a b	a b	a b c	a b c	a b c	a b	a b c d	a b
C. calothyrsus-1																							
Bombana	0 1 0	1 0 0 0	* *	* *	* * 0	* * 0	0 0 1	1 0	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	0 1 0	0 0 1	* * 0	1 0	0 0 1 0	1 0
Barillas	0 1 0	1 0 0 0	1 0	1 0	1 0 0	0 1 0	0 1 0	1 0	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	0 1 0	0 * *	1 0 0	1 0	0 0 1 0	1 0
Cob<n	0 1 0	0 * * 0	* *	1 0	* * 0	* * 0	0 0 1	* *	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	* * 0	0 * *	0 0 1	1 0	0 0 1 0	1 0
Santa MarRa de Jesus	0 1 0	* * * 0	1 0	1 0	1 0 0	0 1 0	0 0 1	* *	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	* * 0	* * *	* * 0	1 0	0 0 1 0	1 0
Patulul	0 1 0	* * * 0	1 0	* *	* * 0	0 1 0	0 0 1	* *	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	* *	* * *	0 * *	1 0 0	1 0	0 0 1 0	1 0
La Ceiba	0 1 0	0 1 0 0	0 1	1 0	* * 0	* * 0	0 0 1	1 0	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	0 1 0	0 * *	* * 0	1 0	0 0 1 0	* *
Maduin	0 1 0	* * * 0	1 0	* *	* * 0	* * 0	0 0 1	* *	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	* *	* * *	0 0 1	1 0 0	1 0	0 0 1 0	1 0
Embu	0 1 0	* * 0 0	1 0	1 0	* * 0	* * 0	0 0 1	1 0	0 1 0	0 1	0 1	1 0	1 0	1 0	0 1	1 0	0 1	* * *	* * *	* * 0	1 0	0 0 1 0	1 0
PAA	**0 1 0**	*** * * 0**	*** ***	*** ***	*** * 0**	*** * 0**	**0 * ***	*** ***	**0 1 0**	**0 1**	**0 1**	**1 0**	**1 0**	**1 0**	**0 1**	**1 0**	*** ***	*** * ***	*** * ***	*** * ***	**1 0**	**0 0 1 0**	*** ***

Population	**Locus**
C. calothyrsus-2	
Fortuna	0 1 0 0 0 1 0 1 0 1 0 1 0 0 1 0 0 0 1 1 0 1 0 0 0 1 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 1 1 0 0 1 0 1 *0* 0 0 1 0 0 1 1 0 0 0 1 0 1 0
Turrialba	0 1 0 0 * * 0 * * 1 0 * * 0 * * 0 0 0 1 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 1 1 0 0 1 0 1 *0* 0 * * 0 0 1 1 0 0 0 1 0 1 0
El Volc<n	0 1 0 0 0 1 0 1 0 1 0 1 0 0 1 0 0 0 1 1 0 1 0 0 0 1 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 1 1 0 0 1 0 1 1 0 0 1 0 1 0 0 1 0 0 * * 1 0
PAA	**0 1 0 0 * * 0 * * 1 0 * * 0 * * 0 0 1 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 1 1 0 1 0 0 1 0 1 1 0 * 0 * 0 * * 0 0 1 1 0 0 * * 1 0**
C. calothyrsus-3	
Cop<n	0 1 0 0 0 0 1 1 0 * * * * 0 0 0 1 * * 0 1 0 0 1 0 1 0 0 1 0 1 * * 1 0 1 0 0 1 * * * 0 0 1 * * 0 1 0 * * * 0 1 0
Meambar	0 1 0 0 0 0 1 1 0 0 1 * * * 0 0 1 * * 0 1 0 0 1 0 1 0 0 1 0 1 0 1 1 0 1 0 0 1 * * * * 0 * * * 0 1 0 * * 0 0 1 0
Santa MarRa	* * 0 0 0 * * 1 0 0 1 * * 0 0 0 1 * * 0 * * 1 0 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 * 0 * * * * * 0 1 0 * * 0 0 1 0
San Ram\n	0 1 0 0 0 * * 1 0 * * * * 0 0 0 1 * * 0 1 0 1 0 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 * * * * 0 * * * 0 1 0 * * 0 0 1 0
La Puerta	0 1 0 0 0 * * 1 0 0 1 * * * 0 0 1 * * 0 * * 1 0 0 1 0 1 0 0 1 0 1 1 0 1 0 0 1 * * * * 0 * * * 0 1 0 * * 0 0 1 0
PAA	*** * 0 0 0 * * 1 0 * * * * * 0 0 1 * * 0 * * 1 0 0 1 0 1 0 0 1 0 1 * * 1 0 1 0 0 1 * * * * * * * 0 1 0 * * * 0 1 0**
C. calothyrsus-4	
Zihuatanejo	* * * 0 0 * * 1 0 * * 1 0 0 0 0 1 1 0 0 * * 0 0 1 1 0 1 0 1 0 0 1 * * * * 0 1 0 * * 1 0 * * * * 0 * 1 0 0 * * * * * 0 0 1

Population	Locus
Cumulative PAA	*** * * * * * * * * * * * * * * *0 * * * * * 0 0 0 0 0 0 0 0 0 0 0 * * * * 0 0 * * * * * * * * * * * * * * * * * * * 1 1 1 1 1 1 1 1 1 1 1 1 1 1**

FIGURE LEGENDS

Fig. 1. Location of Calliandra calothyrsus populations sampled for isozyme analysis and the species native range.

Fig. 2. Phenogram showing isozyme similarities between 17 populations of Calliandra calothyrsus.

Fig. 3. Relationship between rainfall and number of fixed alleles for native populations of Calliandra calothyrsus.