ORIGINAL ARTICLE

AFLP diversity and spatial structure of *Calycophyllum candidissimum* (Rubiaceae), a dominant tree species of Nicaragua's critically endangered seasonally dry forest

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The Central American seasonally dry tropical (SDT) forest biome is one of the worlds' most endangered ecosystems, yet little is known about the genetic consequences of its recent fragmentation. A prominent constituent of this biome is *Calycophyllum candidissimum*, an insect-pollinated and wind-dispersed canopy tree of high socio-economic importance, particularly in Nicaragua. Here, we surveyed amplified fragment length polymorphisms across 13 populations of this species in Nicaragua to elucidate the relative roles of contemporary vs historical factors in shaping its genetic variation. Genetic diversity was low in all investigated populations (mean $H_E = 0.125$), and negatively correlated with latitude. Overall population differentiation was moderate ($\Phi_{ST} = 0.109$, $P < 0.001$), and Bayesian analysis of population structure revealed two major latitudinal clusters (I: 'Pacific North'+'Central Highland'; II: 'Pacific South'), along with a genetic cline between I and II. Population-based cluster analyses indicated a strong pattern of 'isolation by distance' as confirmed by Mantel's test. Our results suggest that (1) the low genetic diversity of these populations reflects biogeographic/population history (colonisation from South America, Pleistocene range contractions) rather than recent human impact; whereas (2) the underlying process of their isolation by distance pattern, which is best explained by 'isolation by dispersal limitation', implies contemporary gene flow between neighbouring populations as likely facilitated by the species' efficient seed dispersal capacity. Overall, these results underscore that even tree species from highly decimated forest regions may be genetically resilient to habitat fragmentation due to species-typical dispersal characteristics, the necessity of broad-scale measures for their conservation notwithstanding.
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INTRODUCTION

The seasonally dry tropical (SDT) forest of Central America ranks among the most threatened forest ecosystems in the tropical lowlands and is even more endangered than the tropical rainforests of Amazonia (Janzen, 1988; Pennington *et al.*, 2006). Formerly extending over very large areas along the Pacific side of Central America, these SDT forest habitats have been so seriously impacted by human activities that nearly all of them have been lost. Historically, the first clearings of these forests probably date back to at least the Archaic Agricultural Period (ca. 6000–5550 BP) and destruction intensified after the 16th century Spanish Conquest (Janzen, 1988; Piperno, 2011). Nonetheless, up to the early-to-mid 20th century, there were still ca. 550 000 km² of SDT forest left, extending in an almost continuous strip along the Pacific side of Central America, from south-western Mexico to north-western Costa Rica, and disjunctively to Panama's Azuero Peninsula (Janzen, 1988; Olson *et al.*, 2001; Griscom and Ashton, 2011). It is now estimated that only 2.0–1.7% of the original SDT forest in Central America escaped deforestation, degradation and conversion into agricultural land and/or pastures, especially between the 1930s and 1970s (Griscom and Ashton, 2011), and only <0.1% of this remaining forest is protected (Miles *et al.*, 2006). This massive habitat loss and disturbance of Central American

SDT forests is well recognised today as a major threat to the region's high biodiversity and endemism (Janzen, 1988; Sánchez-Azofeifa *et al.*, 2005; Linares-Palomino *et al.*, 2011). However, relatively little is known about the impact of this forest degradation on regional-scale genetic structure of constituent tree species.

Especially for outcrossing tree species it has long been assumed that population isolation and decreases in population size associated with anthropogenic forest fragmentation can lead to genetic erosion and increased population differentiation through increased genetic drift, inbreeding and/or reduced gene flow. In turn, such genetic erosion may negatively affect the long-term viability and adaptive potential of remnant populations (Aguilar *et al.*, 2008; Frankham *et al.*, 2010). However, there is increasing evidence that a large number of forest tree species (from both temperate and tropical regions) are able to ameliorate the population genetic consequences of recent habitat fragmentation (reviewed in Lowe *et al.*, 2005, 2015). Potential mechanisms underlying such 'resilience' include a flexible mating system (Ward *et al.*, 2005), extensive gene flow via pollen and/or seed (Bacles *et al.*, 2006) and/or longevity coupled with overlapping generations (Petit and Hampe, 2006; Davies *et al.*, 2010; but for exceptions see, for example, Kettle *et al.*, 2007; Dubreuil *et al.*, 2010).

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In Central America, genetic surveys of SDT forest trees are relatively scarce, highly heterogeneous (for example, in terms of geographical scale of sampling, time or severity of impact, species' life history/ dispersal characteristics) and mostly limited to heavily exploited timber trees (Lowe *et al.*, 2005; Ratnam *et al.*, 2014). Nonetheless, the few broad-scale studies available typically identified low-to-moderate spatial genetic structure (for example, Chase *et al.*, 1995; Gillies *et al.*, 1999; Novick *et al.*, 2003; Poelchau and Hamrick, 2012; but see Cavers *et al.*, 2003, 2013), whereas more fine-scale surveys usually found little or no significant impact of recent habitat loss on genetic diversity parameters (for example, Dawson *et al.*, 1997; White *et al.*, 2002; Fuchs *et al.*, 2003; Lowe *et al.*, 2005; but see Gillies *et al.*, 1999). Overall, these assessments emphasise the need for more individual case studies, as the genetic impacts of fragmentation likely vary by species, geographical scale, current environmental conditions and/or historical context (Lowe *et al.*, 2005; Poelchau and Hamrick, 2013). Moreover, there is a gap left for phylogeographic studies of SDT forest trees, especially in those countries that continued to experience particular high rates of deforestation up to the last few decades, namely Honduras, Guatemala and Nicaragua (Redo *et al.*, 2012; Hansen *et al.*, 2013). Such regional surveys are also important to guideline how remaining SDT forest populations may be effectively conserved *in situ* and optimally sampled for *ex situ* conservation, breeding and restoration programmes (Ceska *et al.*, 1997; Frankham *et al.*, 2010; Diniz-Filho *et al.*, 2012).

Here, we applied dominant amplified fragment length polymorphism (AFLP) markers to assess genetic variation in Nicaraguan populations of *Calycophyllum candidissimum* (Vahl) DC. (Rubiaceae, Ixoroideae, Condamineeae), one of the most common constituents of Central America's degraded SDT forest (Faber-Langendoen *et al.*,

2012). This insect-pollinated and wind-dispersed canopy tree is naturally distributed from southern Mexico through Panama to northern South America (Colombia, Venezuela, Ecuador) and also occurs in the Greater Antilles (Grandtner and Chevrette, 2013; Figure 1a; see Methods section for more details). In Nicaragua, where <1% of the area is covered with SDT forest (Stevens *et al.*, 2001), *C. candidissimum* is valued for its showy inflorescences (for example, sold during religious festivities) and hard, fine-grained wood (used for, for example, house building, carpentry, cabinet work, tool handles, combs, firewood, charcoal; Standley and Williams, 1975; Cordero and Boshier, 2003). Since 1971, it also has received the distinction of being the country's 'National Tree' (Salas, 1993). Apparently for those reasons, this 'multi-purpose' tree has often been left when forests were cleared for agricultural practices, timber, livestock grazing and cotton or other plantations (Barrance *et al.*, 2009; Griscom and Ashton, 2011). Although this habitat degradation has occurred only recently (that is, ca. ⩽ 85 years ago; see above), its presumably negative genetic impact (for example, diversity loss, reduced gene flow) might already be discernible in species with rather short generation times, such as *C. candidissimum*, which only requires ~3–5 years to first flowering under open-grown conditions (R Dolmus/UNAN-Léon; JB Quezada/Universidad Nacional Agraria (UNA), Managua; personal communications). On the other hand, in this wind-dispersed tree, such negative effects might be mitigated through sustained levels of seed flow, but this is something to be tested against molecular data.

Because of its massive habitat loss, *C. candidissimum* essentially lacks primary forest populations that could serve as 'controls' for pre-fragmentation conditions. However, *a priori* expectations about its genetic architecture might be derived from AFLP data available for a
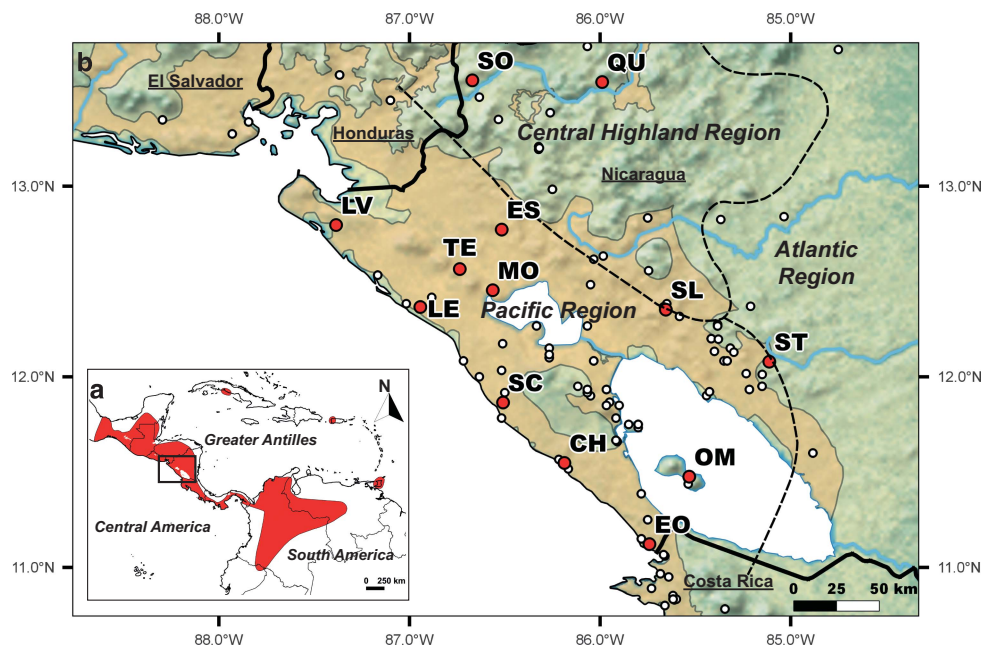
Figure 1 (a) Simplified map of the geographical distribution of *C. candidissimum* in Central America, the Greater Antilles and northern South America based on geo-referenced specimen records ($N=595$) available at TROPICOS (http://www.tropicos.org/Name/27903157 (accessed 20 October 2016)) and the Global Biodiversity Facility (GBIF.org (6th September 2016) GBIF Occurrence Download http://doi.org/10.15468/dl.wc19nm). (b) Geographical locations of 13 populations of *C. candidissimum* in Nicaragua represented in this study for AFLP analysis (red circles), plus additional specimen records ($N=90$) according to TROPICOS/GBIF (small white circles). The light-brown areas represent the original distribution of seasonal dry forest derived from the World Wildlife Fund (WWF) ecoregion dataset (modified from Olson *et al.* (2001)). The dashed lines delimit the physiographic regions of Nicaragua (Pacific, Central Highland, Atlantic) according to Taylor (1963). See Table 1 for identification of population codes.

close relative from the western Amazonian rainforest, *C. spruceanum* (Benth.) Hook.f. ex K.Schum. (Russell *et al.*, 1999). Based on nine populations from the Peruvian Amazon Basin, this study revealed relatively high genetic diversity within and moderate variation among populations, without any major influence of river structure (viz. hydrochory) or geographic distance among sites on genetic distance measures (for example, there was no evident pattern of 'isolation by distance' IBD; Wright, 1943). Overall, these data were indicative of high levels of gene flow among tree populations (mostly via insect-transferred pollen and/or wind-dispersed seed) in a non-fragmented rainforest setting (Russell *et al.*, 1999). For *C. candidissimum* in Nicaragua, therefore, the null hypothesis would be that recent SDT forest fragmentation has resulted in much lower genetic diversity within populations (for example, owing to drift and/or inbreeding), and concomitantly higher genetic variation viz. 'insularization' among populations, in comparison with *C. spruceanum*.

We investigated geographical patterns of AFLP variation in 13 populations of *C. candidissimum* in Nicaragua, which cover much of the country's original dry forest vegetation zone (Figure 1b), and also differ in their degree of disturbance and/or status of legal protection (Table 1). Our major aim was to gain first insights into the relative roles of contemporary vs historical factors in shaping the genetic variation of this emblematic tree species of Central America's highly endangered SDT forest. Concomitantly, we anticipated that the results obtained might help assist future conservation measures that aim at preserving the genetic diversity of this species in Nicaragua. We addressed the following specific questions: (1) to what extent has human-driven landscape fragmentation affected levels of genetic diversity within populations and patterns of gene flow/dispersal (viz. the connectivity) between them? (2) Is there evidence that other factors such as past population dynamics and/or environmental dispersal barriers had a confounding effect on the genetic patterns observed? And (3) what is the conservation value of these populations (for example, are protected ones sufficiently representative of the species' genetic diversity in Nicaragua, or is a broad-scale coverage of conservation measures advisable)?

MATERIALS AND METHODS

The study species

C. candidissimum, also called 'Lemonwood' or 'Madroño', is a diploid, light-requiring pioneer tree (with $2n = 2 \times = 34$ chromosomes; Kiehn, 1995), which grows as tall as 15–20 (30) m (Cordero and Boshier, 2003; Gonzáles-Rivas *et al.*, 2009; Lorence, 2012). Above the glossy leaves, the small, white and bisexual flowers are clustered in large inflorescences (thyrses) in which some flowers bear a large, white-to-greenish calyx lobe or 'calycophyll' (Delprete, 1996). The sweet-scented, protogynous flowers are primarily visited by bees and butterflies (Villegas-Durán *et al.*, 2003; Ramírez-Arriaga *et al.*, 2011; A Dávila-Lara, personal observation) and develop into small, septicidal capsules releasing numerous small (3–5 mm), short-winged seeds that are wind-dispersed (Lorence, 2012; Hilje *et al.*, 2015). Although Bawa (1974) stated that *C. candidissimum* is self-compatible, no concrete experimental evidence was provided due to 'the lack of adequate numbers'. We are unaware of any report indicating that this species regularly reproduces clonally (for example, via root-sprouting).

In Nicaragua, *C. candidissimum* mainly occurs along shady hill sides and along water streams within fragments of secondary and/or riparian SDT forest in the 'Pacific Region' (*sensu* Taylor, 1963) and, more rarely, in isolated stands of semi-deciduous (for example, *Quercus-Pinus*) forest in the 'Central Highland Region' (Harvey *et al.*, 2006; Lorence, 2012; see Figure 1b). The former region has high temperatures and moderate-to-high rainfall, but with strong seasonal drought, whereas the latter has a sub-tropical climate (cf. Taylor, 1963). Throughout its distribution range, *C. candidissimum* is still considered to be locally abundant (Stevens *et al.*, 2001; Lorence, 2012; Kappelle, 2016) and neither listed by the CITES (Convention on Endangered Species; see http://www.cites.org) or the International Union for Conservation of Nature (IUCN, 2016). However, in Nicaragua, it is classified as 'endangered' (threat index '4') according to the Food and Agriculture Organisation of the United Nations (FAO; Roa, 2003).

Plant material and sampling

Between April and August 2013 an extensive survey of the distribution of *C. candidissimum* in Nicaragua was carried out by one of us (AD-L) within the UNAN-León/Department of Biology project 'Conservation and sustainable use of timber and non-timber tree species' in cooperation with the MARENA (Ministry of Environment and Natural Resources)/Nicaragua (MARENA, 2002). Based on this, 13 natural populations were chosen as sampling sites to cover the full geographic range of the species in Nicaragua (see Figure 1b and Table 1): two populations (SO, QU) from the Central Highland Region (407–

Table 1 Information on sample locations for 13 populations of *C. candidissimum* from Nicaragua surveyed for AFLP variation

Code	Locality (sector)[a]	Lat	Long	Alt (m)	Map (mm)	Mat (°C)	Sampled area (ha)	Sampled trees ha^{-1} ($\times 10^{-1}$)	Dist	Prot[b]
SO	Somoto (CH)	13.555	−86.670	648–905	911	22.5	150	7	2	2
QU	Quilalí (CH)	13.546	−85.989	407–535	1346	24.4	61	25	1	2
LV	La Virgen (PN)	12.796	−87.385	23–75	1846	27.0	453	4	2	2
ES	El Sauce (PN)	12.772	−86.516	92–200	1760	26.8	453	4	2	2
TE	Telica (PN)	12.565	−86.736	63–410	1576	26.7	202	8	2	1^N
MO	Momotombo (PN)	12.454	−86.563	62–187	1446	27.7	134	15	1	1^N
LE	León (PN)	12.366	−86.943	1–99	1526	26.8	156	12	2	2
SL	Sn. Lorenzo (PC)	12.352	−85.655	185–321	1189	24.7	152	12	2	2
ST	Sto. Tomás (PC)	12.079	−85.112	125–432	1881	24.1	270	6	2	2
SC	Sn. Cayetano (PC)	11.865	−86.509	66–164	1401	27.4	266	6	2	1^R
CH	Chacocente (PS)	11.548	−86.187	6–86	1702	25.0	79	22	1	1^W
OM	Ometepe (PS)	11.476	−85.532	74–267	1498	26.6	146	13	1	1^U
EO	El Ostional (PFS)	11.122	−85.741	25–56	1658	26.6	116	14	1	2

Abbreviations: Alt, altitude (in metres above sea level); Dist, levels of habitat disturbance: 1, relatively undisturbed, more or less continuous forest; 2, at least partially logged or highly degraded (A Dávila-Lara, personal observation; see also text); Lat, latitude; Long, longitude; Mat and Map, mean annual temperature and precipitation, respectively (derived from www.climate-data.org); Prot, status of legal protection according to IUCN and UNEP-WCMC (2016) and WNBR (2016; only OM): 1, protected; 2, unprotected.
[a]Membership of the populations to latitudinal collection sector and genetic groups: CH, Central Highland; PN, Pacific North; PC, Pacific Central; PS, Pacific South; PFS, Pacific Far South (see Figures 2–4, Supplementary Figure S2, and text for details).
[b]Protection type specified by superscripts: N, Nature Reserve; W, Wildlife Refuge (both IUCN category IV, since 1983); R, Reserve Natural Civil Society (Nicaraguan Foundation for Sustainable Development (FUNDENIC-SOS), since 2009); U, UNESCO-MAB Biosphere Reserve (since 2010).

905 m above sea level), and 11 from the lowland and premontane areas of the Pacific Region (1–432 m above sea level), including one island population (OM) of Lake Nicaragua. Estimated pairwise geographical distances among sampling sites ranged between 25 km (MO–TE) and 287 km (SO–OS) with a mean (± s.d.) of 139 (±63.8) km (data not shown). These 13 sites differed in their degree of disturbance level and/or status of legal protection (Table 1). Five populations (QU, MO, CH, OM, EO) were located in relatively undisturbed, more or less-continuous forest (A Dávila-Lara, personal observation), of which three (MO, CH, OM) were protected; by contrast, all the remaining populations were from at least partially logged or highly degraded areas, and only two of those (TE, SC) were protected. At each site, young leaves were collected from, if possible, up to 20 mature trees that were sampled according to the line transect method of González (2006), with a minimum distance of 100 m between trees to avoid selecting closely related individuals (Gillies *et al.*, 1999; Inza *et al.*, 2012). To ensure sufficient individual sampling, the sizes of the areas surveyed (in total ca. 2638 ha) had to be adjusted accordingly, ranging from 61 ha (for the relatively dense QU population) to 453 ha (for the sparse LV and ES populations) (Table 1). Logistic constraints and the sampling scheme used did not allow for a detailed investigation of the tree density of *C. candidissimum* at any individual site, but it provided the basis for an estimation of the number of sampled trees per hectare at all sites (Table 1). This, together with visual assessments during sampling, allowed for approximating the disturbance level of populations, as indicated above. In total, leaf materials of 250 individuals were collected, dried with silica gel, and stored at room temperature.

DNA extraction and AFLP genotyping

Total genomic DNA was extracted from the dried leaf tissue using a modified cetyltrimethyl ammonium bromide protocol (Doyle, 1991). The AFLP analysis was performed according to Vos *et al.* (1995), with minor modifications as described by Jaros *et al.* (2016) for the use of fluorescent-dye-labelled primers. For a preliminary AFLP screen, we selected one individual each from four populations (LE, CH, OM EO), using 18 selective primer pair combinations with two or three selective nucleotides (data not shown). Based on this, the following primer combinations that produced the best results with respect to polymorphism and clarity of AFLP profiles were chosen for the complete survey: *Eco*RI-AAC/*Mse*I-CA (NED), *Eco*RI-AC/*Mse*I-CTC (VIC) and *Eco*RI-AT/*Mse*I-CTG (6-FAM). Each PCR plate (48 slots) contained two positive controls, one negative control and one blind sample. The selective PCR products were purified using Sephadex G-50 Superfine (GE Healthcare BioSciences, Uppsala, Sweden) following the manufacturer's instructions. AFLP fragments were separated on a MegaBACE 1000 (GE Healthcare Biosciences, Pittsburgh, PA, USA), using the ET400-R-Rox-MegaBACE sizing standard, and manually scored in DAX version 8.0 (Van Mierlo Software Consultancy, Eindhoven, The Netherlands) as described in Bendiksby *et al.* (2011). AFLP products were scored as presence (1) or absence (0), whereby a fragment was considered polymorphic if at least one individual showed a variant pattern. Separate test runs with 16 individuals (representing all 13 populations) were used to obtain an AFLP error rate, calculated as the percentage of differently scored loci over all replicates and the three primer pair combinations (Bonin *et al.*, 2004). In total, 220 individuals were finally available for data analysis because 30 individuals failed to amplify in at least one of the three primer combinations. Accordingly, the mean (± s.d.) sample size was 17.0 (±2.6) individuals per population (see Table 2).

Data analysis

Using the program GENALEX version 6.5 (Peakall and Smouse, 2012), three different measures of genetic diversity were calculated for each locus and then averaged across all loci to represent a single estimate for each population: the mean number of observed alleles per locus (N_A), the percentage of polymorphic fragments (PPF), and Nei's (1987) unbiased gene diversity (H_E) for small sample sizes. In addition, we used the AFLPDAT R package (Ehrich, 2006) to estimate frequency down-weighted marker (DW) values (or 'rarity-indices'), with 95% confidence intervals obtained by bootstrapping over all loci (9999 replicates), and significance tested by a permutation procedure (A Tribsch and D Ehrich, unpublished data) implemented in AFLPDAT (1000 replicates).

Table 2 Summary of AFLP variation for 13 populations (220 individuals) of *C. candidissimum* from Nicaragua

Population code[a]	N	N_A	PPF	H_E	DW[b]
SO	10	1.137	29	0.116 (0.013)	0.573[L]
QU	15	1.208	32	0.111 (0.012)	0.775
LV	20	1.296	41	0.137 (0.013)	0.603
ES	18	1.212	34	0.116 (0.013)	0.632
TE	17	1.212	35	0.122 (0.013)	0.581[L]
MO	20	1.221	34	0.113 (0.012)	0.627
LE	18	1.243	35	0.120 (0.012)	0.648
SL	18	1.261	37	0.127 (0.013)	0.595[L]
ST	16	1.239	35	0.112 (0.012)	0.686
SC	16	1.235	34	0.119 (0.013)	0.615
CH	17	1.332	42	0.141 (0.013)	0.694
OM	19	1.257	36	0.127 (0.013)	0.675
EO	16	1.385	45	0.166 (0.014)	0.999[H]
Species total	220	1.650	65	0.170 (0.012)	—
Species mean	17 (2.6)	1.249 (0.062)	36.1 (4.3)	0.125 (0.015)	0.669 (0.113)

Abbreviations: *DW*, frequency down-weighted marker values; H_E, Nei's (1987) unbiased gene diversity; *N*, number of individuals; N_A, mean number of observed alleles per locus; *PPF*, percentage of polymorphic fragments.
Note: standard deviations are given in parentheses.
[a]Population codes are identified in Table 1.
[b]Significantly low or high *DW* values are indicated by superscripts 'L' and 'H', respectively.

Significantly high vs low values of *DW* may indicate long-term population isolation vs genetic drift and/or founder effects (Skrede *et al.*, 2009). Nonparametric Mann–Whitney *U*-tests were performed to determine whether there was a significant difference in genetic diversity and rarity estimates between populations differing in disturbance level or protection status. Likewise, Spearman's rank correlations were employed to test for a potential relationship between these genetic parameters and various extrinsic factors (latitude, longitude, precipitation, temperature; see Table 1). All these statistical tests were made using the 'Social Science Statistics' online calculators (http://www.socscistatistics.com/).

Genetic structure analysis was performed in STRUCTURE version 2.2 with a Bayesian clustering approach based on a recessive allele model, as applicable for dominant markers (Pritchard *et al.*, 2000; Falush *et al.*, 2007). We run this analysis after pruning markers that proved uninformative (that is, monomorphic) across the entire AFLP dataset (ca. 35%; see Results) and assumed both recent ancestry and some current gene flow among populations (that is, admixture and correlated allele frequencies). The number of clusters (*K*) was set to vary from 1 to 15. For each value of *K*, we performed 10 runs with a burn-in length of 50 000 and a run length of 100 000 Markov chain Monte Carlo replications. To identify the most probable value of *K*, post processing STRUCTURE runs were performed in STRUCTURE HARVESTER version 0.6.94 (Earl and vonHoldt, 2012) to produce graphical files representing the final posterior probability [lnP(*D*)] (Pritchard *et al.*, 2000) and Δ*K* (Evanno *et al.*, 2005) per *K* and repeated run. The CLUMPP program version 1.1.2 (Jakobsson and Rosenberg, 2007) was used to align the membership coefficients from the 10 replicates for each *K* based on the *LargeK Greedy* algorithm.

Analyses of molecular variance (AMOVAs) were performed in ARLEQUIN version 3.5 (Excoffier and Lischer, 2010) to quantify the partitioning of AFLP variance (1) among populations (Φ_{ST}) and within populations; and (2) between regional groups of populations (Φ_{CT}) and among populations within such groups (Φ_{SC}). Significance levels of variance components were evaluated by a permutation approach (1023 replicates). We also used GENALEX to calculate values of Φ_{PT} (an analogue of Φ_{ST}) for all pairs of populations and to infer their significance with 9999 random permutations. Genetic relationships among populations were visualised by subjecting their pairwise Φ_{PT} distances to a principal coordinates analysis (PCoA) in GENALEX. In addition, we constructed a Neighbour-Joining (NJ) phenogram in PHYLIP version 3.69 (Felsenstein, 2005) based on Nei's (1987) unbiased genetic distances calculated using the fragment-frequency approach in AFLPSURV version 1.0 (Vekemans, 2002). The NJ phenogram was midpoint-rooted, with BS (bootstrap support) obtained by resampling with replacement over loci (9999 replicates). Isolation by distance (IBD) was assessed by regressing values of $\Phi_{PT}/(1 - \Phi_{PT})$ against the logarithm (log$_{10}$) of geographical distance (in km) for all pairs of populations (Rousset,

1997), and a Mantel test was employed to evaluate significance with 9999 permutations using IBDWS version 3.23 (Jensen *et al.*, 2005).

RESULTS

Population genetic diversity

For the 220 individuals (13 populations) of *C. candidissimum*, the three AFLP primer combinations resolved a total of 226 unambiguous fragments, ranging from 87 to 411 base pairs (bp), of which 147 markers were polymorphic (*PPF* = 65%; Table 2). Different primer pairs generated a variable number of fragments ('NED': 88; 'VIC': 49; '6-FAM': 89), with an average of 75.3 (±22.8 s.d.) fragments per primer combination. The overall error rate calculated on the basis of 16 replicates (1.16%) is at the lower end of the range reported for other AFLP studies in plant species (Bonin *et al.*, 2004; Poelchau and Hamrick, 2012).

Estimates of genetic diversity (in terms of N_A, *PPF* and H_E) for each population (Table 2) were positively correlated to each other (all $P < 0.001$), but not with the number of individuals (N) sampled per population ($r = 0.29$–0.36, $P = 0.140$–0.675). Values of these diversity parameters were similar across populations and generally low (Table 2). For example, within-population gene diversity (H_E) averaged 0.125 (±0.015 s.d.), with the lowest value (0.111) observed in a Central Highland population (OU) and the highest (0.166) in the southernmost population from the Pacific Region (EO). Although populations in disturbed forest displayed on average slightly lower gene diversity (mean $H_E = 0.121$) than those in undisturbed forest (0.132), this difference was not significant (*U*-test: $Z = -0.732$, $P = 0.465$), and the same was true for N_A and *PPF* ($P = 0.418$ and 0.509, respectively). Similarly, there were no significant differences between populations from unprotected vs protected sites in any of the three diversity parameters (all $P \geqslant 0.660$). Levels of allelic rarity (in terms of *DW*) varied among populations but were generally low, averaging 0.669 (±0.113 s.d.) across all populations (Table 2). Based on permutation tests, one Central Highland (SO) and two Pacific North/Central populations (TE/SL) had significantly low *DW* values, whereas the southernmost Pacific population (EO) had a significantly high value (Table 2). Notably, the proportion of rare markers was on average significantly lower in populations from disturbed (0.617) vs undisturbed forest (0.754; *U*-test, two-tailed significance: $Z = -2.269$, $P = 0.023$). However, no such difference was found between unprotected vs protected populations (0.689 vs 0.638; $P = 0.826$). Correlation analyses revealed a negative and significant relationship between latitude and both N_A ($r = -0.72$, $P = 0.006$) and *PPF* ($r = -0.65$, $P = 0.016$), whereas the same trend was marginally significant for H_E ($r = -0.53$, $P = 0.061$). None of the other factors examined (that is, longitude, precipitation, temperature) showed a significant effect ($P \leqslant 0.05$) on genetic diversity or allelic rarity within populations (data not shown).

Population genetic structure and relationships

In the STRUCTURE analysis, log probabilities of the data [lnP(D)] showed some tendency to plateau at $K \approx 4$, whereas the *ad hoc* statistic ΔK clearly showed the highest likelihood at $K = 2$ (see Supplementary Appendix Figure S1). Based on the latter model, all populations from the Central Highland (SO, QU) and Pacific North Region (LV, ES, TE, MO, LE) were fixed for cluster I ('black' in Figure 2), while cluster II ('grey') was predominant in the three Pacific South populations (CH, OM, EO). By contrast, populations from the Pacific Central Region were differently admixed with clusters I vs II, whereby SL and SC showed higher membership proportions to the northern cluster I and ST to the southern cluster II. At $K = 4$, the Pacific Far South
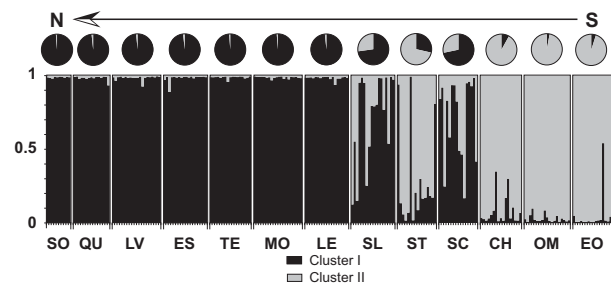
Figure 2 Distribution of the two major AFLP gene pool clusters (I and II) within and among 13 populations (220 individuals) of *C. candidissimum* from Nicaragua as identified by STRUCTURE based on the *ad hoc* statistic ΔK (see also Supplementary Figure S1). The bar-plot displays the assignment of individuals to the two clusters, with the smallest vertical bar representing one individual. The *y* axis presents the estimated membership coefficient (*Q*) for each individual in the two clusters. The *x* axis corresponds to population codes as identified in Table 1. The pie charts at the top represent the average proportion of cluster membership computed across individuals per site. Populations are ordered from left to right according to decreasing latitude (see also Table 1 and Figure 1b).

population (EO) became clearly distinguishable (cluster III), whereas few samples (mostly from QU and LV) were erratically assigned to a fourth 'gene pool', without reflecting a geographical pattern (see Supplementary Figure S2). We therefore retained $K = 2$ as the likely more robust model, albeit without dismissing the genetic distinctiveness of EO (see also below).

The non-hierarchical AMOVA (Table 3) indicated that most of the total genetic variance resided within populations (89.08%) even though the among-population component was highly significant ($\Phi_{ST} = 0.109$, $P < 0.001$). Pairwise Φ_{PT} values (as measures of connectivity between pairs of sites) ranged from 0.005 to 0.229, whereby 93.6% (73/78) were significant ($P < 0.05$) based on permutation tests (see Supplementary Appendix Table S1 and Supplementary Figure S3). The PCoA plot of the first two coordinates (Figure 3a) revealed a more or less continuous north-south alignment of most populations along the first coordinate (44.3% of the total variance), which nonetheless allowed their grouping into: (1) a well-delimited 'North' group (SO, QU, LV, ES, TE, MO, LE); (2) the genetically heterogeneous 'Pacific Central' group (SL/SC and ST); and (3) the 'Pacific South' group (CH, OM). In addition, the second coordinate (15.6%) separated the Pacific Far South population (EO) from the latter group. Hierarchical AMOVA (Table 3) attributed a relatively high percentage of the total variance (9%) to differences among these four population groups, compared with 4.5% among populations, and 86.5% within populations. The PCoA for the first and third coordinates (Figure 3b) suggested a further subdivision of the 'North' group into 'Central Highland' and 'Pacific North' populations. However, the corresponding AMOVA based on five groups resulted in the same amount of among-group partitioning (9%; Table 3). Consistent with the PCoA, the midpoint-rooted NJ phenogram (Figure 4) placed the three populations from the Pacific Central Region (SL, SC, ST) at intermediate branches between the strongly supported 'North' group (BS 95%) and the genetically more distinct southern populations (CH, OM, EO; BS 87%), as would be expected for admixed groups (Verdu *et al.*, 2014). Not unexpectedly, therefore, the Mantel regression of pairwise $\Phi_{PT}/(1 - \Phi_{PT})$ values against the geographical distances among all 13 populations (Figure 5) revealed a positive and highly

Table 3 Non-hierarchical and hierarchical analyses of molecular variance (AMOVAs) for AFLP variation based on several groupings of *C. candidissimum* populations sampled from Nicaragua

Grouping/source of variation	Df	Sum of squares	Variance components	Percentage of total variance	Φ-statistic[a]
Total					
Among populations	12	462.905	1.54	10.9	$\Phi_{ST}=0.109$
Within populations	207	2600.650	12.60	89.1	NC
Four PCoA groups[b]					
Among groups	3	251.205	1.30	9.0	$\Phi_{CT}=0.090$
Among populations	9	211.700	0.65	4.5	$\Phi_{SC}=0.049$
Within populations	207	2600.650	12.60	86.5	$\Phi_{ST}=0.135$
Five PCoA groups[c]					
Among groups	4	290.784	1.30	9.0	$\Phi_{CT}=0.090$
Among populations	8	172.121	0.52	3.6	$\Phi_{SC}=0.040$
Within populations	207	2600.650	12.60	87.4	$\Phi_{ST}=0.126$

Abbreviations: AMOVAs, analyses of molecular variance; df = degrees of freedom; PCoA, principal coordinates analysis; NC = not computed.
[a]All variance components were significant ($P<0.001$) based on 1023 permutations.
[b]Population grouping based on four PCoA units (see Figure 3a), reflecting regional affiliation and gene pool structure: North (SO, QU, LV, ES, TE, MO, LE); Pacific Central (SL, SC, ST), Pacific South (CH, OM), and Pacific Far South (EO).
[c]Respective grouping but with the 'North' further subdivided into Central Highland (SO, QU) vs Pacific North (LV, ES, TE, MO, LE) populations (see Figure 3b).
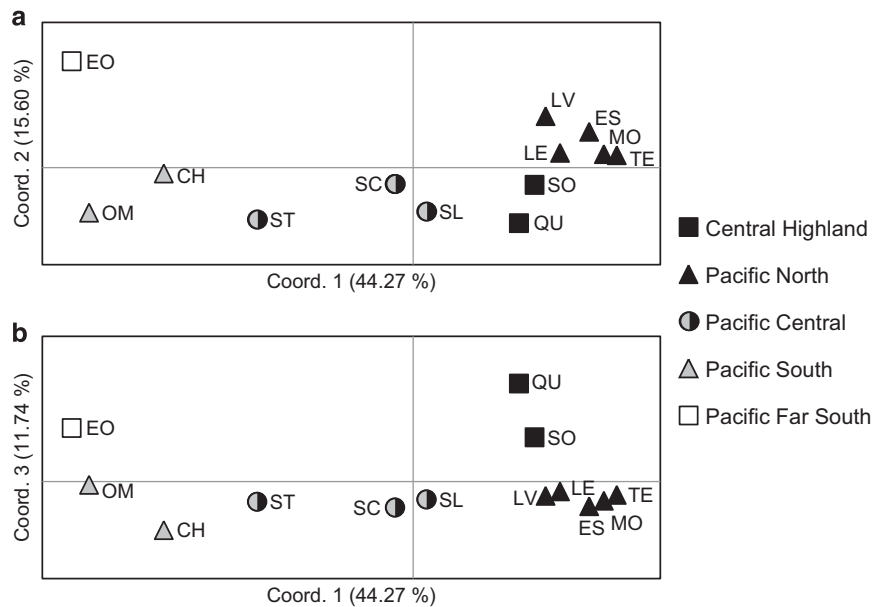
Figure 3 Principal coordinates analysis (PCoA) for 13 populations of *C. candidissimum* from Nicaragua based on pairwise Φ_{PT} values of AFLP data. Plots of (**a**) the first and second, and (**b**) the first and third coordinates. Percentages of total variance explained by each coordinate are noted in parentheses. Population symbols and colours indicate, respectively, regional affiliation (Table 1; Figure 1b) and gene pool (I–III) structure (black, I; grey, II; grey/black, I and II; see Figure 2; and white, III; see Supplementary Figure S2). Note in (**b**) the subdivision between 'Central Highland' and 'Pacific North' populations. Population codes are identified in Table 1.

significant relationship ($r=0.652$, $R^2=0.425$, $P<0.001$), indicating a strong pattern of IBD.

DISCUSSION

Our analysis of AFLP variation within and among 13 populations of *C. candissimum* from fragmented SDT forest in Nicaragua revealed three major findings. (1) Genetic diversity is low in all investigated populations and negatively correlated with latitude. (2) Genetic variation is partitioned into two major regional (latitudinal) clusters and a cline, resulting in an overall moderate level of population

differentiation ($\Phi_{ST}=0.109$). (3) The IBD pattern found may indicate spatially restricted gene flow, but this interpretation requires careful examination. In the following sections, we discuss these results and comment on their implications for conservation.

Low levels of genetic diversity in populations of *C. candidissimum* in Nicaragua

Tropical trees in general, and especially those that are outcrossing, and pollinated and/or dispersed by wind, tend to harbour high levels of genetic diversity at local and regional spatial scales (for example,
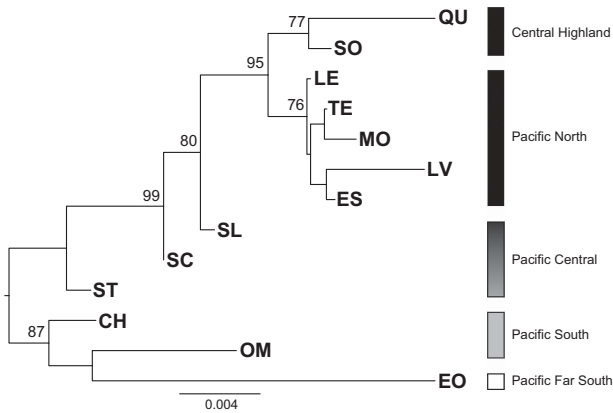
Figure 4 Midpoint-rooted Neighbour-Joining (NJ) phenogram depicting AFLP-derived Nei's (1987) unbiased genetic distances between 13 populations of *C. candidissimum* from Nicaragua. Numbers above branches indicate bootstrap values (≥70%) based on 9999 replicates. Populations are labelled according to their geographic origin. Population codes are identified in Table 1.
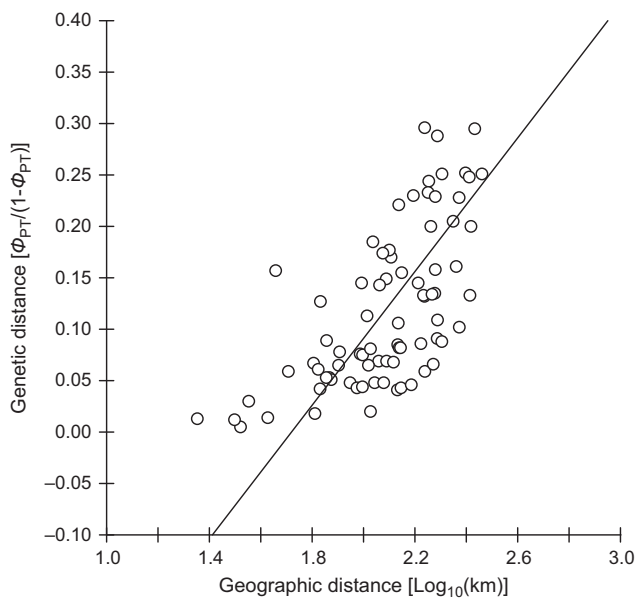
Figure 5 Regression of AFLP-derived $\Phi_{PT}/(1-\Phi_{PT})$ values against the logarithm ($\log_{10}$) of geographic distance (km) for 78 pairwise comparisons of 13 Nicaraguan populations of *C. candidissimum* ($r = 0.651$, $R^2 = 0.425$; Mantel probability (9999 permutations) $P < 0.001$).

Hamrick *et al.*, 1993; Chase *et al.*, 1995; Alvarez-Buylla *et al.*, 1996; Collevatti *et al.*, 2014). However, comparisons across such studies have to be taken with caution owing to discordances in sampling scale, genetic markers and/or data analysis. Nonetheless, our data suggest that populations of *C. candidissimum* in Nicaragua exhibit unexpectedly low diversity (in terms of N_A, PPF, H_E; Table 2). For example, if we focus on values for gene diversity (mean $H_E = 0.125$, range: 0.111–0.166), those are much lower than for populations of the likewise insect-pollinated and wind-dispersed *C. spruceanum* (mean: 0.284, range: 0.249–0.349) surveyed for the same marker system in the Peruvian Amazon Basin (Russell *et al.*, 1999). The same discrepancy emerges if we consider the averages of within-population diversity (*H*)

reported by Kremer *et al.* (2005) for 10 Neotropical outcrossing tree species using AFLPs under the assumption of Hardy–Weinberg equilibrium (mean: 0.23, range: 0.14–0.32) or full selfing (0.24, 0.17–0.38). Although realising that dominant AFLPs cannot provide estimates of inbreeding, we do not consider a highly selfing mating system to be a compelling explanation for the reduced genetic diversity of *C. candidissimum*. First, there is no firm experimental evidence to suggest that this species is truly self-compatible, as surmised by Bawa (1974; see also Methods section). And second, our global estimate of population differentiation ($\Phi_{ST} = 0.109$; Table 3) is much lower when compared to selfers ($\Phi_{ST} = 0.65$; Nybom, 2004) yet more characteristic of a highly outcrossing species (see below). This is in further agreement with field observations and melissopalynogical research indicating that *C. candidissimum* is a highly melliferous tree and important nectar/pollen resource, especially for butterflies (Villegas-Durán *et al.*, 2003; Ramírez-Arriaga *et al.*, 2011).

In the following, therefore, we discuss three alternative factors that may have acted additively and/or synergistically in causing this reduced diversity over time: biogeographic history, Pleistocene range dynamics, and recent anthropogenic deforestation.

First, this reduced diversity may reflect a signature of the species' colonisation of Central America from South America. This scenario gains support from two congruent lines of evidence. (1) Considering *Calycophyllum* DC. in its traditional circumscription (ca. 10–15 spp.; Delprete, 1996; Lorence, 2012), *C. candidissimum* is the only Central American representative of an otherwise South American genus. (2) Although the most recent molecular phylogeny of tribe Condamineeae renders the genus polyphyletic (Kainulainen *et al.*, 2010), it still supports a clade comprised of *C. candidissimum* (the type species) as sister to the South American *C. multiflorum* Griseb. and *C. spruceanum*, which together are likely sister to a clade consisting of genera from mainly South America (that is, the monotypic *Bothriospora* Hook.f., *Dialypetalanthus* Kuhlm., *Wittmackanthus* Kuntz (also found in eastern Panama) and *Alseis* Schott. (ca. 18 spp., three present in Central America)). Although the phylogeny and taxonomy of the entire group requires more work (Delprete and Jardim, 2012), all current evidence seems to indicate that *C. candidissimum* arrived in Central America from South America, perhaps facilitated by the most recent uplift of the Panama land bridge (ca. 3 million years ago), as proposed for many other constituents of the Central American lowland flora (for example, Burnham and Graham, 1999; Novick *et al.*, 2003; Antonelli *et al.*, 2009; Poelchau and Hamrick, 2013; but see Cavers *et al.*, 2013). Thus, the overall depletion of genetic diversity in Nicaraguan *C. candidissimum* could, at least in part, reflect a historical legacy of the species' colonisation from South America (see also Novick *et al.*, 2003). This also concurs with a significant south-to-north trend of declining diversity (in terms of N_A and PPF), consistent with successive ('serial') founder events in the direction of spread (Hewitt, 2004) and the nested (that is, potentially derived) position of northern populations in the NJ phenogram (Figure 4; see also below).

Second, other historical events that may have further contributed to the loss of genetic diversity in Nicaraguan *C. candidissimum* are repeated range shifts in response to the climatic fluctuations of the Quaternary (≤2.5 million years ago), which likely had a major impact on the current genetic structure of many Neotropical plant species (reviewed in Ramírez-Barahona and Eguiarte, 2013). Some authors have hypothesised that the ranges of SDT forest plant species in Central America were not restricted during the cooler and drier periods of the Pleistocene (Piperno, 2006) but rather attained a more continuous distribution (for example, Prado and Gibbs, 1993; Pennington *et al.*, 2000; Novick *et al.*, 2003). This hypothesis seems

intuitively obvious as such plants are tolerant to drought resulting from seasonal rainfall or drought spells during rainy seasons (Pineda-García *et al.*, 2013). In support of this, a recent palaeodistribution modelling of multiple Central American tree species (Poelchau and Hamrick, 2013) found little evidence to suggest that, during the LGM (Last Glacial Maximum; ca. 21 000 years ago; and possibly earlier cold periods), constituents of SDT forest (including *C. candidissimum* but also those of wet-topical forest) retreated into isolated humid refugia (for example, extending from southern Nicaragua to northern Panama), as postulated previously (Brown, 1987); instead, this modelling approach inferred a *decrease* of potential range size since the LGM for at least some of the SDT forest species studied, including *C. candidissimum*. Hence, following its initial colonisation of Central America, this species may have experienced subsequent reductions in historical population sizes owing to inter-/postglacial range contractions, accentuating the loss of diversity through drift.

Finally, recent anthropogenic forest fragmentation may have also contributed to the genetic erosion of Nicaraguan *C. candidissimum*. In general, however, molecular studies to date have found only little evidence for such an effect on levels of genetic diversity in forest trees from the Neotropics and elsewhere, which is usually ascribed to their long generation times relative to the time since deforestation (Lowe *et al.*, 2005; Kramer *et al.*, 2008; Ratnam *et al.*, 2014; but for exceptions see, for example, Gillies *et al.*, 1999; André *et al.*, 2008; Inza *et al.*, 2012). Yet another problem is that measures of genetic diversity differ in their sensitivity to detect such recent impact. For example, while gene diversity (H_E) should be appropriate for assessing long-term (for example, historical) loss of diversity (including common alleles), it often fails to register reductions in diversity in recently bottlenecked populations owing to the loss of rare alleles (Luikart *et al.*, 1998; Aguilar *et al.*, 2008). This is in line with our findings in *C. candidissimum* populations from disturbed vs undisturbed forest in Nicaragua (Table 2): although average levels of H_E (as well as N_A and *PPF*) were reduced in the former, this difference was not significant (for example, $H_E = 0.121$ vs 0.132; $P = 0.248$). By contrast, populations from disturbed forest exhibited on average lower levels of rare makers compared with those from undisturbed sites ($DW = 0.617$ vs 0.754; $P = 0.026$). We therefore conclude that the record of intensified deforestation in Nicaragua (since about the 1930's; Griscom and Ashton, 2011) has not yet impacted overall levels of diversity (N_A, *PPF*, H_E), but has been sufficiently long (compared to the relatively short generation time of our study species) for genetic drift having caused a significant loss of rare alleles in disturbed forest fragments.

Population genetic differentiation, spatial structure and IBD

Despite the highly fragmented landscape occupied by *C. candidissimum* in Nicaragua, we found little evidence for strong genetic structuring across the 13 sampled populations. Although the great majority (93.6%) of estimated pairwise Φ_{PT} values (ranging from 0.005 to 0.229) were significant (Supplementary Table S1), AMOVA revealed an overall Φ_{ST} value of 0.109 (Table 3), which is generally considered to indicate 'moderate' genetic differentiation (Hollingsworth and Ennos, 2004; Freeland *et al.*, 2011). While this value is much lower than the corresponding averages reported for outcrossing (0.27) or wind-dispersed (0.25) species (Nybom, 2004), it is comparable to those of some other insect-pollinated Neotropical trees (for example, *Swietenia macrophylla*, $\Phi_{ST} = 0.126$, Gillies *et al.*, 1999; *Cedrela lilloi*, $\Phi_{PT} = 0.130$, Inza *et al.*, 2012; *Ficus insipida*, $\Phi_{PT} = 0.140$, Poelchau and Hamrick, 2012) and almost identical to that of Amazonian *C. spruceanum* ($\Phi_{ST} = 0.094$; Russell *et al.*, 1999).

Furthermore, although the STRUCTURE analysis (Figure 2) inferred that individuals do cluster into two major population groups that correspond to geographic regions ('North' vs 'Pacific South'), several geographically intermediate 'Pacific Central' populations (SL, SC, ST) had individuals with membership to either the 'North' or 'Pacific South' group. These populations most likely reflect a continuous genetic gradation or admixture of these neighbouring groups (Rosenberg *et al.*, 2005). This interpretation also accords with the among-population relationships detected in the PCoA and NJ analyses (Figures 3 and 4). When coupled with the strong pattern of IBD (Figure 5), these results seem to suggest that population structure in this highly fragmented landscape is still mediated through an equilibrium between genetic drift and gene flow structured by space (Wright, 1943; Slatkin, 1993).

In turn, this would imply ongoing gene flow between presently isolated populations, yet more commonly between neighbouring ones. There are three arguments in support of such a process, recently called 'IBDL' (isolation by dispersal limitation; Orsini *et al.*, 2013). First, our estimates of population differentiation (Φ_{ST}, Φ_{PT}) are probably not strongly biased towards pre-fragmentation conditions (Lowe *et al.*, 2005). Although exact measurements of tree DBH (diameter at breast height) were not undertaken during this study, most sampled individuals had a DBH of ca. 23–80 cm, with only few (mostly from population OM) approaching 110 cm (A Dávila-Lara, personal observation). Assuming a DBH growth rate (mean ± s.d.) of ca. 1.9 ± 1.0 cm per year for *C. candidissimum* (Caughlin *et al.*, 2016), this may indicate that at least a sizable portion of trees we have genotyped were probably not present prior to the onset of intensified deforestation around the 1930's. Second, ensuing loss of SDT forest in Nicaragua must have greatly reduced the effective population size (N_e) of *C. candidissimum* there, leaving migration rate (m) viz. gene flow as the predominant, if not sole factor responsible for counteracting drift to reach, and probably maintain current equilibrium (see also Galbusera *et al.*, 2004). And finally, as typical for a SDT forest tree, the small, winged seeds of this species are released during the dry season (January to April, when mean wind-gust speeds are at their highest in Nicaragua; Stevens *et al.*, 2001), and thus may be dispersed by wind several kilometres, facilitating gene flow beyond the local neighbourhood (Clark *et al.*, 2002). By comparison, pollen flow via bees or butterflies (as main pollinators) might be expected to decay more rapidly with distance from paternal trees, particularly in disturbed tropical forest canopies (for example, Hamrick and Apsit, 2004; Bonebrake *et al.*, 2010; Duminil *et al.*, 2016). In sum, this would suggest that the studied populations still exchange genes (mostly via seed) at a rate dependent upon the geographic distance between habitat fragments.

However, there are two potential caveats to this classical interpretation of IBD. First, estimators of population genetic differentiation (for example, F_{ST}, Φ_{ST}) only represent an integrated indirect measure of the number of migrants across generations (N_em), and therefore must be treated cautiously when used to infer levels of contemporary gene flow (Whitlock and McCauley, 1999). And second, there is increasing awareness that IBD can also be generated through a particular history of 'serial' colonisation and drift (reviewed in Orsini *et al.*, 2013), unless being confounded by environmental conditions and/or local adaptation (Wang and Bradburd, 2014). At first glance, this variant scenario of 'isolation by colonisation' (IBC; *sensu* Orsini *et al.*, 2013) seems to fit the notion that *C. candidissimum* is a Central American colonist (see above). However, under any persisting signature of IBC, one would expect highest levels of genetic differentiation among the most recently colonised populations (Boileau *et al.*, 1992; Slatkin, 1993;

Orsini *et al.*, 2013; Spurgin *et al.*, 2014), but this is not observed. Instead, the potentially derived populations of Nicaraguan *C. candidissimum* from the 'Pacific North' and Central Highland are genetically more similar to each other than to the remainder (see Figures 2–4; Supplementary Table S1), and in spite of their dissimilar elevation ranges and (macro-)climatic conditions (Table 1; Taylor, 1963). Hence, the current data provide no conclusive evidence for IBC or particular environmental (for example, physiographic, climatic) factors influencing spatial population divergence, but also cannot exclude the possibility that other processes than IBDL (for example, historic-demographic, adaptive) underlie the observed pattern of IBD. Disentangling those phenomena, even though difficult, may be achievable through comparisons of correlations between F_{ST} and geographic and ecological distances for, respectively, neutral loci, non-neutral loci and genotypic trait values (see Orsini *et al.*, 2013 for details) and/or coalescent-based approaches to model colonisation (including bottleneck) scenarios (for example, Henn *et al.*, 2012; Spurgin *et al.*, 2014; Dewoody *et al.*, 2015).

Implications for conservation

Measures to conserve the genetic resources of *C. candidissimum* in Nicaragua have been called upon repeatedly (Roa, 2003; Weaver *et al.*, 2003; Noguera-Talavera *et al.*, 2011), mainly because of its socio-economic importance. However, apart from serving as important pollen/nectar resource for insects, this SDT forest tree also functions as foraging, nesting, shelter, and/or resting site (and so on) for a diversity of other animals (for example, howler monkeys; Garber *et al.*, 1999; birds, bats: Harvey *et al.*, 2006). The present data, therefore, have implications for devising conservation plans for Nicaragua's 'National Tree' in the context of a community-based conservation policy also to preserve the integrity of plant-animal interactions (Ruiz-Mallén and Corbera, 2013). Currently, all populations of *C. candidissimum* in this country, regardless of their protection status, are facing risks of losing even more of their already low genetic diversity through reductions in population size, whether due to increased urbanisation/agricultural intensification, especially in the Pacific Region (for example, Redo *et al.*, 2012), or stochastic factors (for example, natural or man-made fires; de Dixmude, 2001), with likely detrimental consequences for their long-term fitness and adaptation (Frankham *et al.*, 2010). In fact, when compared with their protected counterparts (TE, MO, SC, CH, OM), the eight populations analysed here from outside nature reserves do not harbour significantly lower genetic diversity (Table 2), thus emphasising the need to extend close monitoring, preventive action, and *in situ* conservation to them as well. In view of limited resources, and based on our Φ_{ST} estimate of 0.109, one might argue to focus *ex situ* sampling efforts (for example, for provenance trials) on just two populations, representing > 95% of the total genetic variation (that is, $1 - (\Phi_{ST})^2 = 0.99$; see Ceska *et al.* (1997)). However, our data show that Nicaraguan *C. candidissimum* is comprised of two major regional genetic clusters (I and II), and a remarkable cline (between I and II; Figure 2), calling for a broad coverage of both *in situ* and *ex situ* conservation measures. Hence, 'North' (I) vs 'Pacific South' (II) populations should be valued separately under conservation programmes (for example, by avoiding human assisted gene exchange between them), whereas those from the Pacific Central Region (SL, SC, ST) likewise warrant special attention, as they are likely stepping-stones for natural gene flow between these groups. The same should apply to the southernmost EO population, given its unusually high diversity/rarity (Table 2), divergent gene pool (detectable at $K = 4$; Supplementary Figure S2), and location in a transition zone between SDT and seasonal moist forest (Taylor, 1963; Olson *et al.*, 2001).

Notably, too, this divergence occurs in a region (southern Isthmus of Rivas/north-western Costa Rica) where previous large-scale phylogeographic studies have registered genetic discontinuities in dry-forest trees (*Cedrela odorata*: Cavers *et al.*, 2003, 2013; *Brosimum alicastrum*: Poelchau and Hamrick, 2012) and various animals (for example, ants, bats, birds, reptiles; reviewed in Bagley and Johnson, 2014). Hence, only a wider sampling could clarify whether EO represents a genetically unique population or just delimitates the northernmost edge of a perhaps more widespread southern lineage.

Conclusions and research gaps

This study has assessed for the first time genetic variation for a SDT forest tree in Nicaragua. When compared with *C. spruceanum* from continuous Amazonian rainforest (Russell *et al.*, 1999), Nicaraguan *C. candidissimum* exhibits lower genetic diversity and, despite similarly moderate overall population differentiation (Φ_{ST}), stronger geographical structure (IBD). We propose this reduced diversity more likely reflects population history (that is, the species' colonisation from South America; Pleistocene (inter-/postglacial) range contractions) rather than recent human impact, whereas the strong pattern of IBD is probably best explained by 'dispersal limitation' rather than 'isolation by serial colonisation' (*sensu* Orsini *et al.*, 2013). The spatial genetic patterns observed thus conform to expectations of a subdivided meta-population (Wright, 1943) in which drift–migration equilibrium is still maintained by ongoing gene flow, possibly owing to the species' efficient seed dispersal capacity. Overall, these results underscore that even tree species from one of the world's most decimated forest regions may be genetically resilient to habitat fragmentation due to species-typical dispersal characteristics that ensure nearest-neighbouring population connectivity and viability (Bacles *et al.*, 2006; Kramer *et al.*, 2008; Lowe *et al.*, 2015). However, future research is required to arrive at more conclusive evidence about the recurrent and historical factors shaping genetic variation in *C. candidissimum*.

First, our inference of ongoing gene flow should be re-examined using approaches that can provide more direct estimates of pollen and seed movement at both local and regional scales, such as parentage and/or paternity analyses based on nuclear microsatellite loci (for example, Gerber *et al.*, 2014). Second, such co-dominant markers are also critical to examine whether inbreeding (due to selfing and/or sib mating) is increased by the small size and isolation of these populations (that is, in terms of F_{IS}), and whether levels of genetic diversity differ between adult trees and seedlings (for example, Davies *et al.*, 2010; Duminil *et al.*, 2016). Third, experimental and field data are required to assess whether the genetic processes leading to the loss of rare alleles, as found in some of the populations studied here, also contribute to decreased survival, reproductive output and/or progeny fitness of individual trees, as indicated by a recent monitoring of *C. candidissimum* in Southwest Nicaragua (Noguera-Talavera *et al.*, 2011). Fourth, more integrated approaches using both neutral and non-neutral genetic markers in relation to spatial and environmental factors would be useful to test for local adaptation in *C. candidissimum* across its entire range in Central/northern South America (Orsini *et al.*, 2013; Wang and Bradburd, 2014). And finally, this Neotropical tree and its allies should provide a promising study model for phylogenetic and phylogeographic research as indicated by the biogeographic and population demographic scenarios we have invoked here.

DATA ARCHIVING

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3274n.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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