

Regional scale genetic structure within two Central American tree species the influence of geography, biology and geological history

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Abstract: We examine spatial genetic structure occurring at a regional scale for two widely distributed tree species from Central America, *Vochysia ferruginea* and *Cedrela odorata*, and speculate on some of the geographic, edaphic and geological factors that may be responsible in a continent that has a complex geological history and is rich in natural resources. For *V. ferruginea*, the central mountain ranges of Costa Rica coincide with a major genetic differentiation and are probably acting to maintain this genetic split by reducing gene flow. A major genetic differentiation within Costa Rica is also apparent for *C. odorata*, for both nuclear and chloroplast markers. However, this split coincides with habitat type (dry vs. moist), not with geographic features and appears to be a case of ecotypic differentiation or possibly cryptic speciation. A cpDNA phylogeographic analysis of *C. odorata* across Central America reveals that the strong genetic/ecotypic differentiation continues across a large portion of the region. The pattern of cpDNA variation is discussed within the context of species colonisation and extinction. However, a scenario that involves separate and repeated colonisation of Central American regions from diverse sources most closely fits the present pattern. Future work to examine cpDNA variation across the Caribbean and South America should shed more light on this issue. Finally the importance of such genetic data for prioritising the management of forest genetic resources is discussed.

Introduction

Mesoamerica is recognised as a global biodiversity hotspot due to the high number of species found in the region (e.g. approximately 24 000 plant species) and the current high rates of habitat destruction. The pressure on primary vegetation is extreme, and only 20% of the original extent of primary vegetation now remains (Myers *et al.* 2000). Commercially valuable timber species have been particularly heavily impacted due to targeted extraction combined with habitat loss. Conservation efforts are now being made, but protected land is not

always coincident with the distribution of threatened species. Furthermore, the importance of managing genetic resources of such species is also generally poorly appreciated. In particular, there is little information on the evolutionary history of species or the extent and structure of genetic diversity for species that cover a wide geographic area.

To develop realistic management strategies for the genetic resources of key species, it is essential to have an understanding of the structure of genetic diversity within existing populations. It is also essential to know how the dynamics of gene flow will affect variation. The structure of genetic variation can be influenced by many factors including: biology (breeding system, seed and pollen dispersal mechanisms and regeneration ecology), geography (topographic features which may reduce gene flow) and history (geology and biogeography). Studies that adopt a comparative approach, for species with similar ranges, will allow the identification of those factors that influence genetic structure.

Presented here are preliminary data on regional-scale genetic structure within two tree species from Costa Rica chosen for their economic importance and wide ranging distributions: *Cedrela odorata* and *Vochysia ferruginea*. In addition, *C. odorata* was subject to a cpDNA phylogeographic analysis across much of its Central American range to examine patterning of genetic variation at a continental scale.

Materials and methods

Cedrela odorata L. is a fast growing, light demanding tree, distributed from Mexico (26° N) to Argentina (28° S). It is monoecious, pollinated by small bees and wasps and has wind dispersed seed. It is a commercially valuable timber and is threatened by deforestation and over exploitation. Its modern day distribution is patchy, and individuals are often left as remnant trees on farmland established on cleared forest areas. It is capable of tolerating fairly dry conditions (1200-2000 mm rainfall p.a.) and grows up to 1200 m above sea level. *C. odorata* is recognised in the Costa Rican National Conservation Strategy and is listed by the IUCN as vulnerable.

Vochysia ferruginea Mart. is a typical canopy tree of lowland forest, and distributed from Nicaragua to Brazil. It is a pioneer species and recolonises cleared land, forming dense secondary stands. It prefers moist conditions (2500-4000 mm rainfall p.a.) and grows up to 800 m above sea level. The species tolerates high soil concentrations of aluminium and iron and is fast growing, which together with its good timber qualities, makes it ideal for reforestation or reclaiming degraded land. Individuals bloom synchronously in populations but

its breeding system has been variably reported as outcrossing to strict autogamy, including cleistantheric pollination. It has hermaphroditic flowers that are pollinated by birds and insects (mainly larger bees) and has wind dispersed seed.

Collections

For both species, collections comprised up to 20 individuals from each of ten Costa Rican populations (Table 1, 2). In each case, collections were from the full geographic range of the species within Costa Rica (Fig 1). For *C. odorata*, additional collections were made throughout Central America, with a total of 29 populations sampled from Mexico, Guatemala, Honduras, Nicaragua, Costa Rica and Panama. Each of these populations consisted of between 5 and 20 individuals (Table 1, 2).

PCR-RFLP screening of chloroplast DNA

Full screening is still underway but preliminary results are presented here. DNA extraction, PCR, restriction digestion and gel electrophoresis methods followed those of Demesure *et al.* (1995). Preliminary analysis to identify polymorphism within each species involved screening a single individual from each population for several primer/enzyme combinations. In total, 79 primer/enzyme combinations were analysed, and the combined length of PCR products covered approximately 38,000 bp of the chloroplast genome. For *C. odorata*, where five cpDNA haplotypes were identified, genetic relationships between haplotypes were resolved using minimum spanning methods.

AFLP analysis

Ten Costa Rican populations of up to 20 individuals of *C. odorata* were further screened for AFLP variation (Table 1) using the protocol of Vos *et al.* (1995). Genomic DNA was digested using the enzymes *MseI* and *EcoRI* and preamplified using primers *MseI*+AC and *EcoRI*+0. The selective amplification step used primers *MseI*+ACAG and *EcoRI*+CC. Genetic distances between populations were analysed using Nei's distance algorithm based on population fragment frequencies (POPGENE, Yeh 1997). Pairwise genetic distances were clustered using the Neighbour-Joining algorithm (PHYLIP, Felsenstein 1993).

Table 1. List of collection sites, locations, sample sizes for cpDNA and AFLP studies, and cpDNA PCR-RFLP haplotypes for *Cedrela odorata*.

Country	Population	Lat.	Long.	RFLP1	RFLP2	RFLP3	RFLP4	RFLP5	AFLP
				Central	Southern	Northern	Guatemala	Panama	
Costa Rica	Puriscal	9.93	-84.29	20	0	0	0	0	13
Costa Rica	Horizontes	10.74	-85.59	20	0	0	0	0	13
Costa Rica	Canas	10.2	-84.95	20	0	0	0	0	13
Costa Rica	Hojancha	10.09	-85.37	20	0	0	0	0	17
Costa Rica	Palo Verde	10.35	-85.35	20	0	0	0	0	1
Nicaragua	Masatepe	11.9	-86.14	20	0	0	0	0	
Nicaragua	Ometepe	11.49	-85.49	20	0	0	0	0	
Nicaragua	La Trinidad	12.99	-86.23	20	0	0	0	0	
Nicaragua	Wabule	12.88	-85.68	20	0	0	0	0	
Honduras	Comayagua	14.15	-87.62	20	0	0	0	0	
Honduras	Meambar	14.83	-88.1	20	0	0	0	0	
Honduras	La Paz	14.42	-87.05	20	0	0	0	0	
Honduras	Taulabe	14.83	-88.1	20	0	0	0	0	
Costa Rica	Jimenez	10.21	-83.61	0	20	0	0	0	14
Costa Rica	Talamanca	9.63	-82.85	0	20	0	0	0	13
Costa Rica	Upala	10.79	-85.03	1	19	0	0	0	14
Costa Rica	Pacifico Sur	8.54	-82.85	0	20	0	0	0	16
Costa Rica	Perez Zeledon	9.33	-83.65	0	20	0	0	0	10
Panama	Gualaca	8.58	-82.24	0	0	0	0	20	
Panama	Las Lajas	8.2	-81.86	0	16	0	0	4	
Panama	San Francisco	8.24	-80.98	0	0	0	0	20	
Guatemala	Los Esclavos	14.25	-90.28	0	0	4	1	0	
Guatemala	Tikal	17.23	-89.62	0	0	5	0	0	
Guatemala	San Jose	17.18	-89.86	0	0	5	0	0	
Guatemala	El Idolo	14.43	-91.38	0	0	5	0	0	
Mexico	Bacalar	18.29	-89.15	0	0	20	0	0	
Mexico	Calakmul	18.45	-88.32	0	0	20	0	0	
Mexico	Zona Maya	19.36	-88.02	0	0	20	0	0	
Mexico	Escarcega	18.4	-90.9	0	0	20	0	0	

Table 2: List of collection sites, locations, sample sizes and cpDNA PCR-RFLP haplotypes for *Vochysia ferruginea*.

Country	Population	Lat.	Log.	RFLP1	RFLP2
Costa Rica	La Marta	9.76	-83.68	17	
Costa Rica	Penjamo	10.33	-84.48	9	
Costa Rica	Tirimbina	10.39	-84.14	13	
Costa Rica	Perez Zeledon	9.36	-83.69		20
Costa Rica	Coto Brus	8.9	-83.09		20
Costa Rica	Pindeco	9.2	-83.47		19
Costa Rica	Brasillea	11.03	-85.36	10	
Costa Rica	Osa	8.72	-83.49		11
Costa Rica	Puriscal	7.17	-89.36		13
Costa Rica	Cano Negro	10.95	-84.71	-	-

Results

For both species, two chloroplast haplotypes were found within Costa Rica, and distributions are plotted in Figure 1. For *V. ferruginea*, no within population variation was found, whereas for *C. odorata*, one population (Upala) was found to be polymorphic. *Vochysia ferruginea* exhibited significant intraspecific genetic differentiation that coincided with the central mountain range of Costa Rica, i.e. individuals from Pacific populations possessed a different haplotype to those from the Atlantic. By contrast, the major genetic differentiation for *C. odorata* did not correlate with the mountain barrier, but closely corresponds to habitat type (wet and dry; Figure 3).

Preliminary AFLP results for *C. odorata* are presented in Figure 2. Of 52 loci scored using a single primer combination, 40 were polymorphic. The pattern of variation is similar to that found for cpDNA analysis, as populations from drier regions (NW Costa Rica) clustered together and were strongly differentiated from those of moist regions. Further differentiation occurred between populations within the moist regions, and populations on the Atlantic side of the mountain chain (Jimenez, Talamanca and Upala) were distinct from those on the Pacific side (Pacífico Sur and Perez Zeledon).

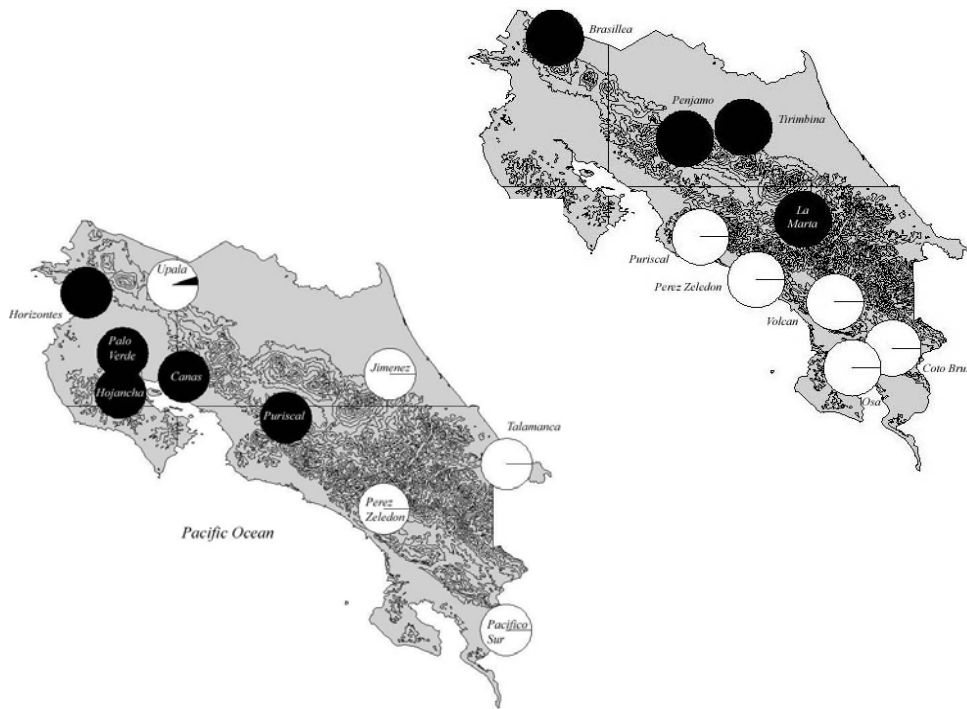


Figure 1. Distribution of cpDNA haplotypes for *C. odorata* (left) and *V. ferruginea* (right).

Finally the analysis of cpDNA variation across the range of *C. odorata* in Central America identified a total of five cpDNA types. Their location and minimum spanning relationship can be seen in Figure 3. Three commonly occurring haplotypes were found, restricted to three specific areas: 1. Dry regions of Honduras, Nicaragua and Costa Rica; 2. Moist regions of Costa Rica and Panama; and 3. Mixed habitat types in Mexico and Guatemala. Two other, rare haplotypes, 4 and 5, were found in Guatemala and Panama respectively (Figure 3). The minimum spanning relationship between the haplotypes is shown in the inset of Figure 3. Of the common haplotypes, haplotypes 1 and 2 were most similar (3 mutations differentiated them) but haplotype 3 was most similar to haplotype 2 (5 mutations) rather than the geographically proximate haplotype 1 (8 mutations). Of the less common haplotypes, 5 was most similar to 2, and 4 to 3, with one mutation differentiating each pair.

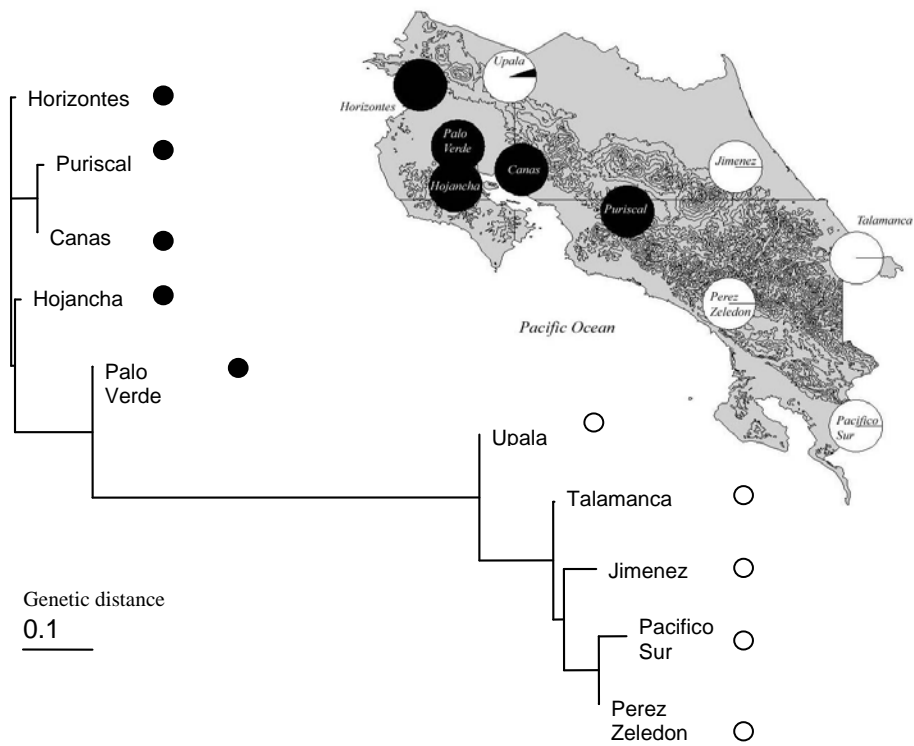


Figure 2. Neighbouring joining dendrogram based on shared presence of 52 AFLP fragments for 10 Costa Rican populations of *C. odorata*. Map indicates location of sampled populations.

Discussion

Geographic partitioning of haplotypes

Significant genetic differentiation within Costa Rica was uncovered for both species. For *V. ferruginea*, genetically differentiated populations were split by the central mountain ranges of the country; the Cordillera de Guanacaste, Cordillera de Tilaran, Cordillera Central and Cordillera de Talamanca (Figure 1). For *C. odorata*, AFLP analysis of moist region populations also demonstrated genetic differentiation of populations that were split by the central mountain ranges. Several reasons can be postulated to explain such genetic differentiation including isolation and drift caused by the uplift of the mountain

chain (at least 1 million years ago), or colonisation of the Pacific and Atlantic areas from different source populations. However, at this stage it is difficult to identify the reasons for the genetic differentiation of Atlantic and Pacific populations. What we can say is that this central mountain range represents a significant barrier to gene exchange and is probably responsible for the maintenance of some of the genetic differentiation within both species.

Ecological partitioning of haplotypes

In contrast to the situation found for *V. ferruginea*, the major intraspecific genetic differentiation within *C. odorata* coincides closely with rainfall and habitat, not the mountain barrier (Figure 1 and 3). This split is apparent from both cpDNA and AFLP analysis and indicates significant differentiation. This significant genetic divergence was also noted by an earlier RAPD study (Gillies *et al.* 1997) and individuals growing within the two habitats have significant morphological and physiological differences (Newton *et al.* 1997). Atlantic region populations have a higher growth rate, redder newly emergent leaves and smoother bark than those from the northern Pacific region (Guanacaste).

Significant differentiation associated with habitat is also seen for other species within this region. For example, the genus *Swietenia* exhibits two forms in Costa Rica, differentiated according to habitat, and in this case classified as separate species. *Swietenia macrophylla*, a widely distributed, moist forest species, inhabits Atlantic and southern Pacific areas similar to the 'wet' form of *C. odorata*, whereas, *S. humilis* is only found in the dry areas of Guanacaste and further north along the Pacific Coast of Central America. The work of Chase *et al.* (1995) on *Cordia alliodora* also demonstrates a significant genetic split along an Atlantic/Pacific axis.

The reasons for this strong habitat-correlated genetic divergence are more difficult to elucidate. Certainly there is some evidence for strong selection against the dry forms when they are reared in the wet regions and vice versa (Adrian Newton pers. comm.). The AFLP data show no evidence of hybridisation between the ecotypes, even in populations where they are sympatric, such as Upala. This suggests the presence of a post-zygotic breeding barrier maintaining the genetic difference between forms. However, further work will need to be done, particularly in the area of sympatry, to examine fertility and selection pressures on inter-ecotype crosses. At the moment these genetically and morphologically distinct populations are recognised as two ecotypes of the same species, but there may be good arguments to revise the taxonomy and recognise the two forms as separate species. Indeed the two forms may have been isolated for a considerable period of time, as evidenced by the considerable differentiation for neutral characters, and are only now in

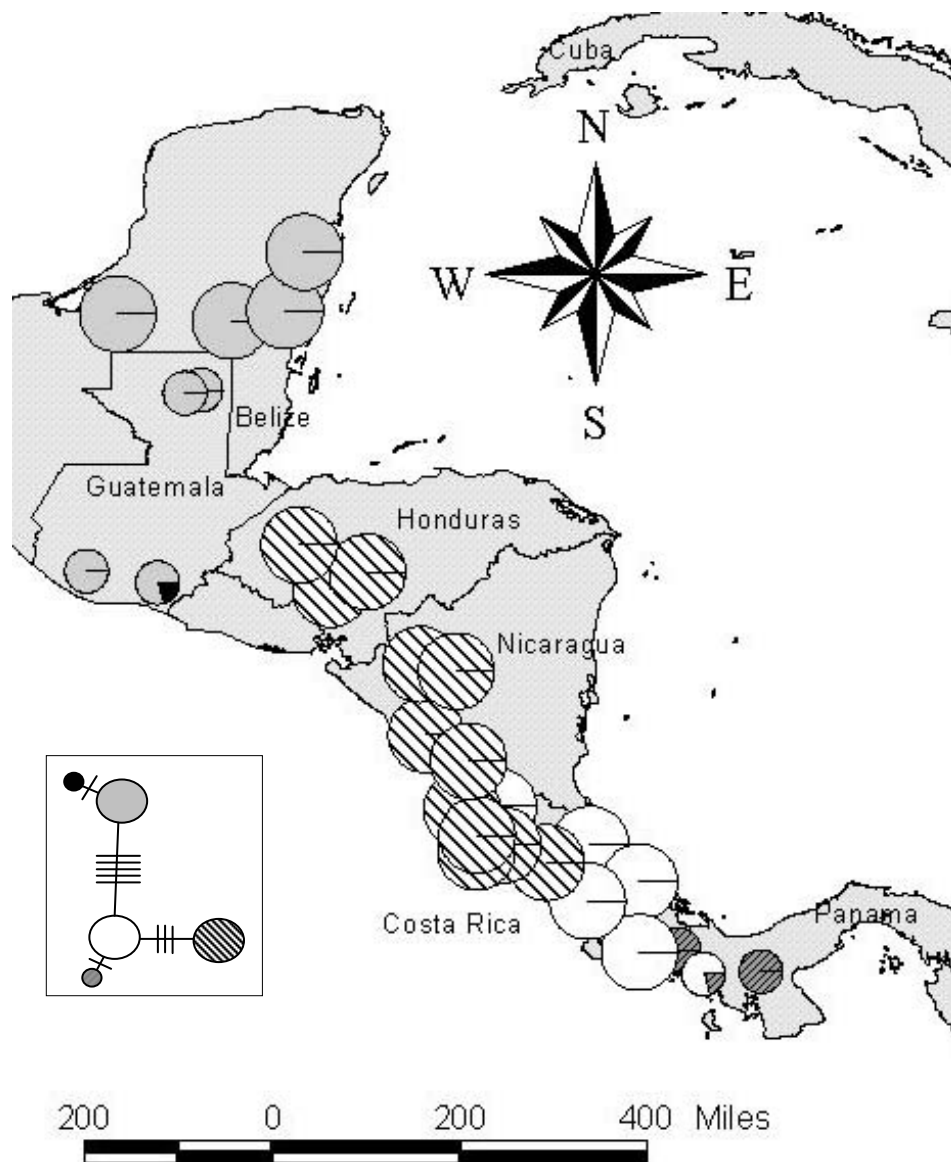


Figure 3. Distribution of five cpDNA haplotypes found within *C. odorata* samples collected from across Central America. Mean annual precipitation of locations is indicated. Primer enzyme combinations used to distinguish haplotypes and populations sample sizes are presented in Table 1. In this figure, Haplotype 1 = central, Haplotype 2 = southern, Haplotype 3 = northern, Haplotype 4 = Guatemalan and Haplotype 5= Panamanian.

secondary contact after evolution in allopatry. In such a case the two forms should be more appropriately described as separate species.

Range wide phylogeography, influence of species history

The range of sampling of *C. odorata* was extended across most of its Central American distribution. The correlation between habitat type and genetic differentiation observed within Costa Rica is maintained throughout the continental sample (Figure 3) where haplotype 1 occurs in dry regions within Costa Rica, Nicaragua and Honduras and haplotype 2 occurs only in moist regions of Costa Rica and Panama. A third major haplotype (3) was found within Mexican and Guatemalan populations. It was found predominantly in wet regions but also occurred in dry areas of the Yucatan Peninsula. In addition, two rare haplotypes were found. Haplotype 4 was restricted to a single individual from Guatemala and haplotype 5 was found in central Panamanian populations. The distribution of the most common haplotypes and their genetic relationship is somewhat puzzling. Populations in Mexico possess a haplotype that is most closely related to the southern type (haplotype 1) and not the haplotype (2) found in proximate Honduran populations. One explanation for such a pattern could be that the Mexican material represents a different colonisation history to other Central American populations. Pollen of *Cedrela* species has been recorded from the Yucatan Peninsula in the early Miocene (20 MY; Graham 1999), well before the formation of the Isthmus of Panama. From this time, either the Caribbean Islands, which were located much closer to the continental masses of Central and South America, or an island arc along the line of present day Central America, could have provided routes by which *Cedrela* species could have established in the Yucatan. Following the rise of the Isthmus and the joining of Central and South America via Panama approx. 3 million years ago, further colonisations from different sources could have established *C. odorata* in southern Central America, creating the boundaries between different genetic types that we observe in the present day distribution. This idea will need further testing and will include sampling of the Caribbean Islands and South American populations. In particular we will need to establish the origin of this genus and potential source and colonised regions. This work is on-going and the hypothesis will be tested alongside other scenarios that include extinctions and recolonisations during past climatic changes.

Conclusions and application to genetic resource management

The structure of genetic diversity within a species range is established by complex interactions between the biology of the species and its geographic and environmental history. However, it is not yet possible to provide predictive estimates of how each of these factors contribute to establishing genetic structure. Indeed in a survey of the isozyme literature, Hamrick *et al.* (1992) were only able to explain 34% of the variation in the data set by combining all biological and ecological characters. Thus individual case studies are still required when developing appropriate genetic resource management strategies for target species.

For the species examined here, the geographic regions of the Pacific and Atlantic need to be recognised as genetically distinct for *V. ferruginea*. For *C. odorata*, the major differentiation between the ecotypes is of most importance to be considered but also the significant subdivision across the mountain barrier within the range of the wet ecotype should be recognised. Further work is required to establish the taxonomic relevance of these differentiations. Whilst these are specific case studies, similar patterns of variation for other species are also seen within the region. It is certain that the particular pattern of topography and environment of the area has had an influence on many species. When considering other Costa Rican target species for which no genetic data is available it would be appropriate to include Atlantic and Pacific, and wet and dry regions when designing any preliminary sampling strategy.

The influence of species history is particularly difficult to predict. Whilst interesting observations are possible from the study of *C. odorata* it is not possible to use them to predict the evolutionary history of the other species in this study. The study of *C. odorata*, is however, informative with regard to the management of genetic resources, and gives insight into the time that the different areas of the species range may have been isolated and their potential connectivity.

It is clear from these case studies that many factors cause significant genetic differentiation within species, and need to be taken into consideration during the planning of genetic resource management. Such divergence can be due to a complex interaction of geographic, adaptive and historical processes (Newton *et al.* 1999), particularly in a geologically and environmentally dynamic region like Central America.

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Questions received after oral presentation and answers given

*How confident are you that the populations are native and that the pattern of cpDNA variation detected, particularly within *C. odorata*, is due to natural, not human mediated processes?*

The possibility of human influence on genetic structure for such phylogeographic studies can never be entirely ruled out. Central America has been the location of fairly extensive human civilisations for at least the last two thousand years. However, whilst forest clearance and large scale harvest of wood from natural populations has almost certainly occurred, there is little to indicate ancient movement of seed or other silvicultural plantation in the area. So whilst human populations may have caused a reduction in genetic diversity and associated bottlenecks within exploited populations it is doubtful that the more widely distributed ancient civilisations (e.g. Maya and Aztecs) would have moved significant amounts of *C. odorata* germplasm around the whole of Central America. Human civilisations from central and southern areas of the continent were not nearly so extensive as those in northern regions and tended to be geographically restricted, again limiting the chance of germplasm transfer. More recent colonial settlers harvested wood from natural forests but there are few records of transplantation of material. When sampling, we tried to select the oldest trees (large diameter) from intact forest blocks to minimise the chance of sampling recently planted material. Thus we believe we have minimised the chance of sampling allochthonous material, however, this possibility cannot be fully ruled out. Another way to address this question is to ask what would happen to the genetic structure if there had been extensive movement and transplantation of material? One would expect considerable mixing of haplotypes in Central American populations, which was not found.

Why speculate a Caribbean route of colonisation to explain the existence of haplotype 3 in the Yucatan Peninsula and not an extinction of this haplotype at other central locations?

This question is still under study and hopefully a wider sample of material from the Caribbean and South America will be examined. Both scenarios will be considered during the interpretation of results, but we view the former explanation the most parsimonious at present.

*Why not investigate the influence of forest refugia and glacial climatic cycling on the continent-scale pattern of genetic variation with *C. odorata*?*

Cedrela odorata is able to grow under very dry conditions and is often found in open woodland and pasture. It is not restricted to moist forest conditions, and thus climatic drying during the last glacial maximum is not likely to have had such a large impact on the distribution of *C. odorata* compared to moist forest species. However, a rare haplotype (4) was found in one of the Guatemalan populations, the location of which coincides with a major forest refugium postulated by Toledo (1981). Thus the influence of recent climatic change on the distribution of genetic variation within *C. odorata* cannot be ruled out, but we believe that more ancient colonisation and migration processes are responsible for establishing the major patterns of cpDNA variation that we find today. Certainly *C. odorata* would not be a good choice of species if one wanted to locate the postulated glacial forest refugia in Central America using molecular methods. Further phylogeographic studies of moist forest-dwelling species, sensitive to climatic change, will no doubt shed light on the influence of recent climatic changes on forest distribution in Central America.

*Could there have been a selective sweep within *C. odorata* for cpDNA type?*

The strong association of cpDNA type with habitat type for haplotypes 1 and 2 could be due to a selective sweep process, however, this is a difficult process to prove. The existence of other morphological and physiological characters associated with habitat suggests selection acting on nuclear genes, but this question will need further investigation.