SPECIAL ISSUE

Genetic evidence of Quaternary demographic changes in four rain forest tree species sampled across the Isthmus of Panama

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ABSTRACT

Aim We examined and compared population genetic structure in a suite of four co-occurring Panamanian tree species and performed coalescent-based analyses of demographic history to evaluate hypotheses of tropical vegetation change during the Last Glacial Maximum (LGM).

Location Isthmus of Panama.

Methods Nuclear microsatellite variation was assayed in multiple populations (1179 trees, 21 locations, 6–13 locations per species) in *Jacaranda copaia* (Bignoniaceae), *Luehea seemannii* (Malvaceae), *Simarouba amara* (Simaroubaceae) and *Symphonia globulifera* (Clusiaceae). Population structure was analysed using F_{ST}-based statistics and a Bayesian clustering approach (BAPS). Bayesian coalescent methods (MSVAR) were used to infer demographic histories.

Results High levels of genetic diversity were found in all of the species (H_E range, 0.56–0.79). *Jacaranda copaia* and *L. seemannii* showed lower F_{ST} and fewer Bayesian clusters across similar spatial scales than did *S. globulifera* and *Simarouba amara*. For each species examined, the current effective population sizes (N_e) are much lower than ancient N_e, within all inferred BAPS demes. In light-demanding pioneer species *J. copaia*, *L. seemannii* and *S. amara*, estimates of the number of generations since the bottleneck events overlap with the end of the LGM (median site posterior estimates ranged from 16 to 19 ka) while *Symphonia globulifera* estimates are consistent with earlier population declines (median 202 ka) in the early Pleistocene and late Pliocene.

Main conclusions The wind-dispersed deciduous species *J. copaia* and *L. seemannii* showed lower F_{ST} and spatially extensive demes, while the animal-dispersed evergreen *Symphonia globulifera* and *Simarouba amara* showed spatially restrictive demes and higher F_{ST}. Each deme examined shows evidence of historical bottlenecks. For three of the four species which are also light-demanding pioneer species, the mean estimated time and 95% highest posterior density of the bottleneck events coincides with the end of the LGM. These results suggest that these species have undergone historical bottlenecks as a result of reduced forest cover during the Pleistocene and provide evidence of shared demographic histories among co-occurring tropical forest trees.

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INTRODUCTION

Understanding the role of past climate change in determining current biodiversity patterns can inform predictions of future responses to anthropogenic climate change (McLachlan *et al.*, 2005; Petit *et al.*, 2008; Hoban *et al.*, 2010). However, this research programme poses significant challenges, particularly in tropical forests with high species diversity (Hannah & Lovejoy, 2007; Corlett, 2011). Chief among the challenges is the individualistic nature of the species that inhabit tropical forests (Colinvaux *et al.*, 1996a; Stewart *et al.*, 2010), where hundreds to thousands of coexisting tree species can vary considerably in ecological niche dimensions (e.g. Condit *et al.*, 2002; Engelbrecht *et al.*, 2007). Because fossil pollen rarely provides species-level taxonomic resolution, multi-taxon population genetic analysis is an alternative approach for inferring historical vegetation change and understanding the behaviour of individual species.

Although geological and climatic events have shaped species diversity in tropical tree communities over deep time (Morley, 2000; Pennington & Dick, 2010), the recent events of the Quaternary are more likely to have left a recoverable genetic imprint within modern tree populations. The Pleistocene was a dynamic period of rapid climate change at mid and high latitudes (Hofreiter & Stewart, 2009), where most population and phylogeographic studies have been performed. It is widely accepted that many temperate tree species underwent successive range shifts, local extinctions and recolonizations in response to Pleistocene climatic changes (Dynesius & Jansson, 2000; Davis & Shaw, 2001), which impacted the distribution of population genetic diversity in these species (Petit *et al.*, 1997, 2003).

Due in part to a scant fossil pollen record and overall higher species richness, less is known about how Neotropical tree communities responded to Pleistocene climate changes than for mid and high latitudes. The Neotropics, like much of the Earth, were cooler and drier during the Last Glacial Maximum (LGM), with estimates of temperatures 5 °C cooler than present (Colinvaux *et al.*, 1996b), and vast regions of what is now considered moist forest (forest that experiences seasonal drought) and wet forest (little to no seasonal drought) were occupied by species that are associated with dry forests, savanna and grasslands (see references in Pennington *et al.*, 2000). Historical vegetation models for Central America range from persistence of moist forest (Poelchau & Hamrick, 2013) to severe restriction of moist and wet forests to refugial areas (Brown, 1979; Aide & Rivera, 1998; Piperno, 2007). Most reconstructions accept that cooler and drier temperatures led to a displacement of moist and wet forest species by seasonally dry forests, open woodlands and savannas, which would be expected to reduce and/or fragment the ranges of species associated with these forests (Brown, 1979; Pennington *et al.*, 2000). However, palaeo-niche modelling of several moist forest species suggests that many of these forests probably persisted during the LGM and may in fact have contracted during the Holocene due to the reduction in land area resulting from rising sea levels (Poelchau & Hamrick, 2013). Palynological evidence from the Brazilian Amazon also calls into doubt the idea that Pleistocene climate changes caused a shift to more arid forest types in the area (Colinvaux *et al.*, 2000).

Did tropical forest tree species undergo expansion into and out of refugia or persist in the landscape through glacial cycles? Gene coalescent simulations can be used to test for changes in historical effective population sizes (N_e) and provide estimates of timing of these events. Coalescent approaches therefore allow us to differentiate between different scenarios of vegetation change (Kuhner, 2009). For example, if populations were not affected by changing climate, we should expect to find evidence of constant N_e through time and a comparatively long time to coalescence of intraspecific genealogies. If species persisted within small refugial areas and subsequently expanded after the LGM, on the other hand, we would expect to find genetic evidence of population bottlenecks (and/or expansions) and a short time to coalescence.

In order to develop a genetic data set to compare with niche-modelling and palynological studies geared towards understanding Panamanian forest history (Piperno *et al.*, 1990; Bush *et al.*, 1992; Poelchau & Hamrick, 2013), we examined population genetic structure and demographic history in four unrelated tree species that coexist sympatrically within many wet and moist forests in Panama (Fig. 1). These four tree species differ in several key life-history characteristics, including dispersal mechanism and drought deciduousness, traits that could have influenced their demographic response to past climate change. We were guided by the following questions.

1. Do the focal co-occurring species share similar patterns of genetic diversity, spatial genetic structure and demographic history?

2. Do populations within central Panama show evidence of population stability, increases or decreases in effective population size since the Pleistocene?

MATERIALS AND METHODS

Study areas and sampling

Our research was conducted primarily in lowland and mid-elevation forests in central and western Panama (Fig. 1a). A rainfall gradient exists in central Panama with Pacific forests receiving less than 2000 mm of rainfall each year compared to up to 4000 mm on Caribbean coasts (Pyke *et al.*, 2001). These forests contain more than 1000 tree species with exceptionally high rates of species turnover (beta diversity) largely due to the rainfall gradient and to a complex mosaic of soil types (Condit *et al.*, 2002). Leaf samples were collected from mature primary and secondary closed canopy forests (our aim was *n* = 30 leaves per species per site). The final sample size depended upon the natural abundance of species at each site (see Appendix S1 in Supporting Information). The
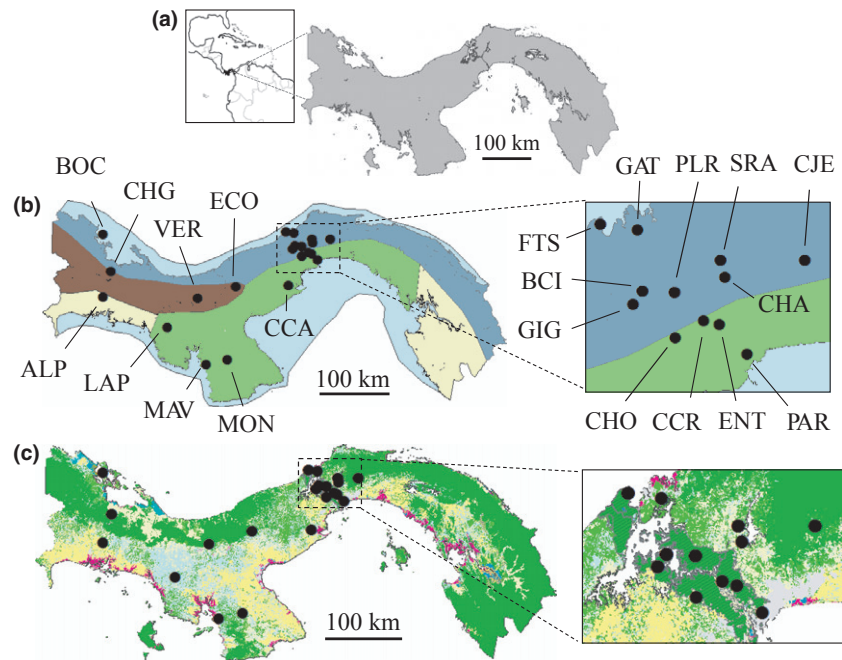
Figure 1 (a) Map of sampling locations for four Neotropical tree species in Panama. (b) Pleistocene vegetation reconstruction of Panama modified from Piperno (2007) with sampling locations and abbreviation codes in black (see Appendix S1 for full details). Coastlines exposed by reduced sea levels (light blue), unbroken moist forest (blue) and mountain zones higher than 1500 m (brown), thorn woodland, low scrub and wooded savanna vegetation (green) and dry forest (light yellow). (c) Current vegetation from the year 2000 (scale 1:250,000; Instituto Geografico Nacional Tommy Guardia, Panama). Primary and secondary mature forest (dark green), disturbed forest (light green), agricultural area (light yellow), mangrove forest (pink), flooded forest (blue), and pioneer forest (light blue). Sampling locations are the same as in panel (b).

sampling was largely carried out along transects with at least 50 m separation between sampled adult individuals in an effort to avoid sampling closely related individuals. All four species were collected at each site when they occurred in sufficient abundance to collect leaves from 25 to 30 individuals per site. Therefore, our sampling effort reflects the distribution and abundance of each study species across central and western Panama.

Quaternary vegetation change in Panama

Based on palynological records (Piperno & Jones, 2003; Piperno, 2006) the Pacific coast of Central America was considerably drier during the LGM than today, and was probably dominated by dry forests, low woodlands, thorn scrub and savanna vegetation (Fig. 1b). The wetter Caribbean coast has been hypothesized to have harboured vegetation similar to the evergreen and semi-evergreen forest present today and therefore may have served as a refuge for moist forest species (Brown, 1979; Poelchau & Hamrick, 2013). The end of the glacial period and onset of the Holocene brought warmer and wetter conditions to the area (Bush *et al.*, 1992), which presumably would have increased the area available for wet and moist forest adapted species. However, rising sea levels during this time also reduced the overall terrestrial area of the region (Fig. 1b).

Focal species

Appendix S1 provides a comparison of all four study species, including sampling locations and published sources of microsatellite markers.

Jacaranda copaia (Aubl.) D. Don (Bignoniaceae) is a wind-dispersed, deciduous, long-lived pioneer tree also found in old growth forests from Bolivia to Belize. In Panama, *J. copaia* is restricted to wet and moist forest types and is less common on the Pacific slope. *Jacaranda copaia* is pollinated by large bees and generalist insect pollinators (Maués, 2008) and produces small wind-dispersed seeds that are occasionally transported for long distances (Jones *et al.*, 2005; Jones & Muller-Landau, 2008; Wright *et al.*, 2008). Mating system studies have shown that *J. copaia* is predominantly outcrossed (James *et al.*, 1998; Maués, 2008). It is strongly shade intolerant and requires large tree-fall gaps to regenerate (Wright *et al.*, 2003).

Luehea seemannii Triana & Planch (Malvaceae) is a light-demanding, deciduous to semi-deciduous (Croat, 1978; Meinzer *et al.*, 1999) canopy tree common in wet to dry primary and secondary low-elevation forests in Panama and Costa Rica. It is pollinated by a large variety of generalist insects (Haber & Frankie, 1982), and produces many small (6–10 mm long) wind-dispersed seeds (Croat, 1978). The mating system has been reported as self-incompatible (Bawa *et al.*, 1985) and partially to fully self-compatible (Haber &

Frankie, 1982). *Luehea seemannii* typically requires tree-fall gaps in which to regenerate (Wright *et al.*, 2003) but it can also reproduce clonally through stump sprouting.

Simarouba amara Aubl. (Simaroubaceae) is an evergreen species found in lowland wet and moist forests of upper Central America to the western Amazon and Guiana Shield. In Panama, it is scarce on the drier Pacific slope, where it is restricted to mid-elevation habitats. *Simarouba amara* is dioecious, insect pollinated and dispersed primarily by vertebrates (birds and large mammals), which can move seeds several hundred metres (Hardesty *et al.*, 2006). *Simarouba amara* requires intermediate- to high-light environments found in tree-fall gaps for growth and reproduction and is common in secondary forests (Wright *et al.*, 2003).

Symphonia globulifera L.f. (Clusiaceae) is a shade-tolerant evergreen tree that is common in upland areas but is also associated with seasonally flooded forests (Jeník, 1967; Baraloto *et al.*, 2007). It is pollinated primarily by hummingbirds and perching nectar-feeding birds (Gill *et al.*, 1998). The *c.* 3-cm greenish-brown berries are consumed by birds, monkeys and bats. The genus *Symphonia* is represented by a distinct fossil pollen type, which is often used to identify rain-forest vegetation in stratigraphic samples (Dick *et al.*, 2003).

Laboratory methods and microsatellite analyses

Details of DNA extraction, primer design and polymerase chain reaction (PCR) conditions are given in the publications listed in Appendix S1. For each population, we calculated the number of alleles per locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E) and the fixation index (F_{IS}) using the program GENALEX 6.1 (Peakall & Smouse, 2006). We tested for isolation-by-distance (IBD) using a stepping-stone model of migration (Rousset, 1997), under the null hypothesis of no correlation between pairwise estimates of $F_{ST}/(1-F_{ST})$ and $R_{ST}/(1-R_{ST})$ (where F_{ST} and R_{ST} are measures of population genetic structure with R_{ST} assuming a stepwise mutation model) and the logarithm of geographic distances between populations. The IBD analysis was performed with GENALEX 6.1 using a Mantel test with 10,000 permutations.

We complemented the F_{ST} and R_{ST} based analyses with a Bayesian clustering approach implemented in the software BAPS 5.4 (Corander *et al.*, 2003, 2008; Corander & Marttinen, 2006). A mixture analysis was used to cluster sample locations and the geographic coordinates of each location (i.e. a spatially explicit model) were used as prior information to determine the posterior probability of different number of demes (K) until the maximum number of diverged groups (i.e. the number of demes sampled) was reached. Overall, the spatially explicit model performed better than a uniform non-informative prior, probably due to the limited number of microsatellite loci used in this study. Each analysis was carried out independently 10 times for each species to account for model uncertainty. Once the most likely value of K was identified based on the posterior probability (PP), an admixture analysis across inferred populations was

conducted using 100 iterations, and the significance of individual admixture coefficients was compared with those of 200 simulated non-admixed reference individuals.

Evidence for past demographic changes across the focal species was tested using a Bayesian coalescent approach implemented in the MSVAR 1.3 software program (Beaumont, 1999; Storz & Beaumont, 2002). A simulation study has shown that this method is effective at detecting past population declines or expansions and generally outperforms other methods of detecting population bottlenecks from microsatellite data (Girod *et al.*, 2011). MSVAR 1.3 uses a hierarchical model where the parameters are allowed to vary among loci (Storz & Beaumont, 2002) and it provides multi-locus posterior distribution estimates of N_1 (ancient effective population size), N_0 (recent effective population size), μ (mutation rate) and $X_a = g_a$ (generation time) $\times T_a$ (number of generations since expansion/decline). Because undetected population structure can generate false bottleneck signals (Chikhi *et al.*, 2010), we fitted separate MSVAR models of population expansion/decline for each of the inferred demes (i.e. BAPS clusters).

Generation time (g_a) refers to the average age of successful reproduction, which is a difficult parameter to estimate for tropical trees as neither age nor fecundity data are readily available. We applied three broad estimates of g_a for our focal species, representing an approximate age to first reproduction (25 years) and two older ages (100 and 175 years) in order to place broad confidence intervals around our estimates of absolute time. Because g_a is a constant in the MSVAR analysis, the time since demographic change in absolute years (X_a) is determined by the posterior estimates of the number of generations since decline (T_a).

We used uninformative lognormal priors as follows: means of the prior distributions were set to 3, 3, –4 and 4; and SD equal to 3, 3, 1 and 3 for N_1, N_0, μ and T_a parameters, respectively. Hyper-prior lognormal distributions were set thus: mean equal to 6, 6, –3 and 8; SD equal to 2, 2, 0.25 and 2; hyper-prior of mean equal to 0, 0, 0 and 0; and hyper-prior of SD equal to 0.5, 0.5, 0.5 and 0.5 for N_1, N_0, μ and T_a parameters, respectively. Each Markov chain Monte Carlo (MCMC) was run for 6×10^9 iterations and thinned at each 5×10^4 interval. The R package BOA 1.1.7-2 (Smith, 2007) was used to estimate the mean, standard deviation and 95% highest posterior density (HPD) limits after merging the five chains for each run and discarding the first half of the iterations of each chain as burn-in (Smith, 2007). BOA was also used to assess convergence among five chains using multivariate potential scale reduction factor statistics (Gelman & Rubin, 1992; Brooks & Gelman, 1998). Multivariate estimates indicated that all chains converged in the MSVAR analysis.

RESULTS

Genetic structure

Using 22 microsatellite loci (6 in *J. copaia*, 6 in *L. seemannii*, 5 in *Simarouba amara*, and 5 in *Symphonia globulifera*), we

genotyped 1179 individual trees from 21 different locations across central and western Panama (Appendix S1). The microsatellite diversity of the four species ranged from moderate ($H_E = 0.56$ for *Simarouba amara*) to high ($H_E = 0.79$ for *J. copaia*). The average number of alleles per locus ranged from 9.44 (SE 0.43) in *J. copaia* to 5.289 (SE 0.42) in *S. amara*. The inbreeding coefficient (F_{IS}) was significantly greater than zero in *S. amara* and *Symphonia globulifera* (Table 1).

All species showed significant global F_{ST} with the lowest estimate in *J. copaia* ($F_{ST} = 0.058$, SE = 0.003) and the highest in *Simarouba amara* ($F_{ST} = 0.163$, SE = 0.021, Appendix S2). Most pairwise values of F_{ST} were significant after Bonferroni corrections (Appendix S2). *Jacaranda copaia* showed high F_{ST} between central Panama and Bocas del Toro (BDT), but little differentiation within central Panama. *Luehea seemannii* was similar to *J. copaia* in that the central Panama populations were strongly differentiated from the BDT populations and other populations in western Panama, but no significant differentiation was detected among sampling locations within central Panama (Appendix S2). *Simarouba amara* showed a more complex pattern of differentiation with high differentiation between some populations (Montuoso (MON) versus BDT, $F_{ST} = 0.57$), but a more complex pattern of differentiation between western and central Panama, a result previously reported in Hardesty *et al.* (2010). *Symphonia globulifera* showed less overall population differentiation than *Simarouba amara* using *F*-statistics, but its patterns were also complex, with high population differentiation occurring between populations sampled on Barro Colorado Island (BCI) and Fort Sherman (FTS) ($F_{ST} = 0.38$) and between FTS and Pipeline Road (PLR) ($F_{ST} = 0.31$). All these three sites are within 24 km of each other, and the result is in strong contrast to lower differentiation observed in other populations located greater distances apart. There was no evidence of significant IBD in any species except for *L. seemannii*, and only for F_{ST} ($r^2 = 0.338$, $P < 0.001$, data not shown).

Bayesian analysis of population structure

Bayesian clustering results are consistent with F_{ST} results (Fig. 2, Appendix S2). *Jacaranda copaia* and *L. seemannii* showed lower structure across central Panama than *Simarouba amara* and *Symphonia globulifera*, as evidenced by fewer demes. The BAPS analysis revealed a higher posterior probability of $K = 2$ for *J. copaia* out of six locations sampled with a spatially extensive deme in the Panama Canal watershed. None of the *J. copaia* individuals showed significant admixture (Fig. 2). *Luehea seemannii* ($K = 3$ of 13 locations) also had a spatially extensive deme in the canal watershed and separate inferred demes on the Pacific slope of western Panama and Bocas del Toro province. Within the canal watershed, seven individuals showed significant admixture with two other inferred demes. *Simarouba amara* had a posterior probability of $K = 7$ out of nine locations, with a

Table 1 Mean number of individuals of four Neotropical tree species genotyped per locus per population (n), number of microsatellite loci used in the analysis, average number of alleles (A_n), effective number of alleles (A_E), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), proportion of genetic variation in subpopulation relative to the total (F_{ST}) and proportion of genetic variation in the individual relative to the total (F_{IT}).

Species	Populations	Loci	n	A_n	A_E	H_O	H_E	F_{IS}	F_{ST}	F_{IT}	K
Jacaranda copaia	6	6	27.6 (0.57)	9.44 (0.43)	5.45 (0.31)	0.79 (0.019)	0.793 (0.012)	0.003 (0.29)	0.058 (0.003)	0.06 (0.029)	2
Luehea seemannii	13	6	29.603 (0.906)	5.538 (0.223)	2.748 (0.113)	0.576 (0.021)	0.583 (0.018)	0.012 (0.012)	0.079 (0.014)	0.089 (0.019)	3
Simarouba amara	9	5	29.867 (1.191)	5.289 (0.42)	2.914 (0.232)	0.504 (0.038)	0.560 (0.032)	0.117 (0.104)	0.163 (0.021)	0.267 (0.079)	7
Symphonia globulifera	8	5	28.55 (0.598)	9.025 (0.515)	4.697 (0.261)	0.718 (0.030)	0.756 (0.016)	0.064 (0.075)	0.116 (0.010)	0.170 (0.073)	8

Standard errors are in parentheses. K is the number of clusters identified in a Bayesian analysis of population structure using BAPS.

spatially extensive deme encompassing FTS, Santa Rita Ridge (SRA), and Cerro Jefe (CJE) in central Panama. Fifteen individuals from BCI and from the extensive cluster including FTS, SRA and CJE showed significant admixture with other demes. *Symphonia globulifera* showed the most population differentiation among the species studied here, with each of the eight sampled locations forming a different deme. Five individuals showed significant admixture among different demes.

Current and ancient N_e and demographic change

Full posterior estimates for the MSVAR analysis are given in Appendix S3. When ancient N_e is estimated and compared to current N_e to estimate the ratio of current to ancient population size (N_0/N_1), the MSVAR analysis demonstrates that all inferred demes show strong evidence of historical population bottlenecks with $N_0 < N_1$ (Fig. 3a). Most estimates for N_1 and N_0 do not overlap across demes within species, except for *S. globulifera*.

The number of generations since population decline (T_a) was also examined across demes and revealed differences among *S. globulifera* and the other three focal species (Fig. 3b). *Symphonia globulifera* experienced an order of magnitude more generations since population decline on average than the other species, with some *S. globulifera* demes having estimates of population decline that overlap with the end of the Pliocene. Mean estimates of time to population change (X_a) across all demes range from 486 ka considering a 25-year generation time (g_a) to 3450 ka (175 g_a), the latter concurrent with the closure of the Isthmus of Panama.

Jacaranda copaia, *L. seemannii* and *Simarouba amara* have broad overlap in the number of generations since decline with the greatest number of generations since decline estimates placed in the mid to late Pleistocene and with HPD estimates for most demes overlapping the end of the LGM, regardless of the 25- or 175-year estimate of g_a. Mean absolute timing of bottleneck events across demes for *J. copaia* ranged from 16 ka (25 g_a) to 115 ka (175 g_a). Mean esti-
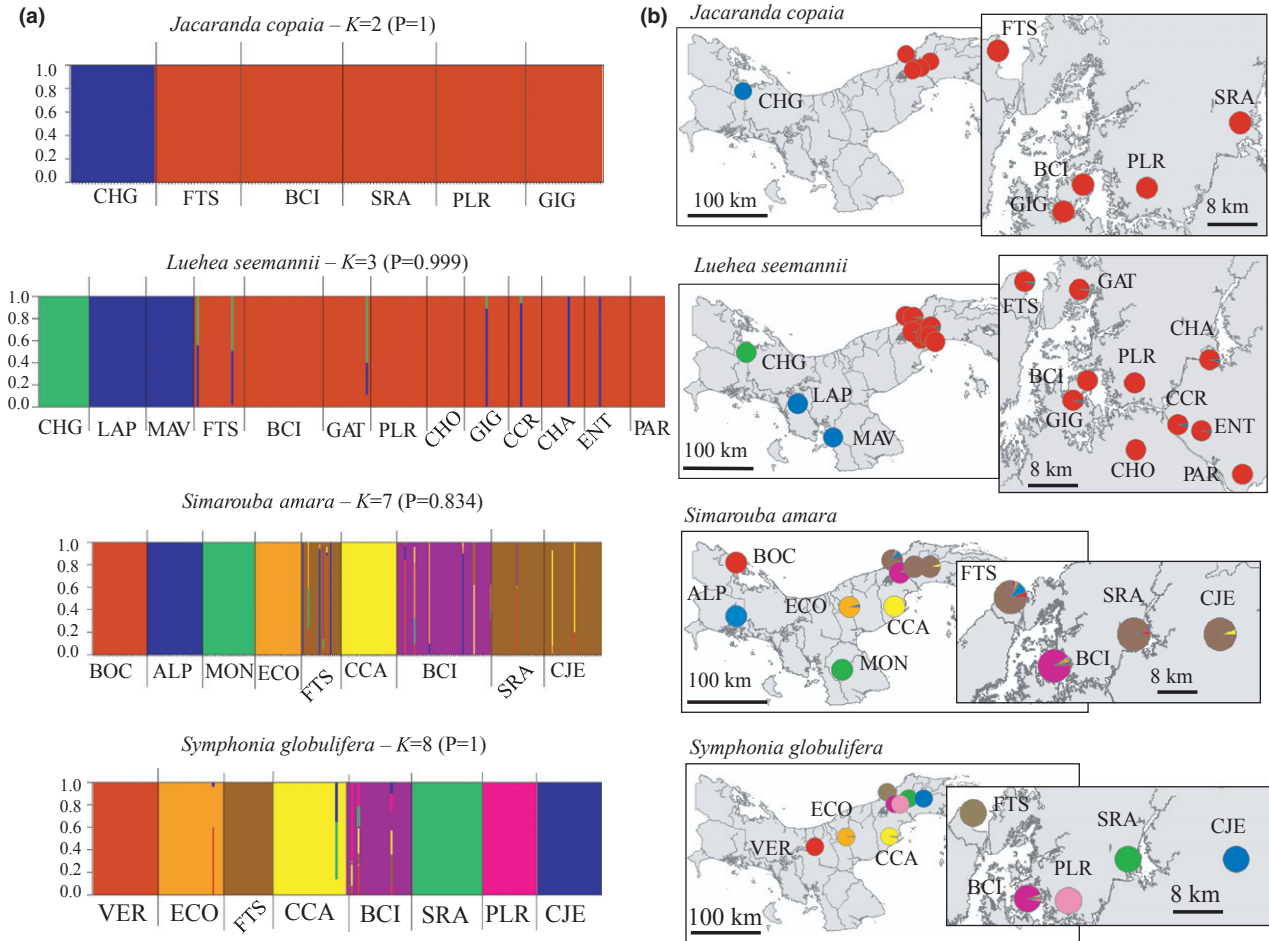

Figure 2 Bayesian clustering analysis and admixture analysis at a group level (i.e. clustering of sample locations) of each of four Neotropical tree species included in the BAPS analysis. (a) Within each species, *K* is the most likely deme number (the value with the highest posterior probability, *P*). The different colours in each vertical line represent the assignment of individuals to their respective population. (b) Pie charts represent the proportion of individuals of different demes inferred by BAPS. Sampling location codes are provided in Appendix S1.
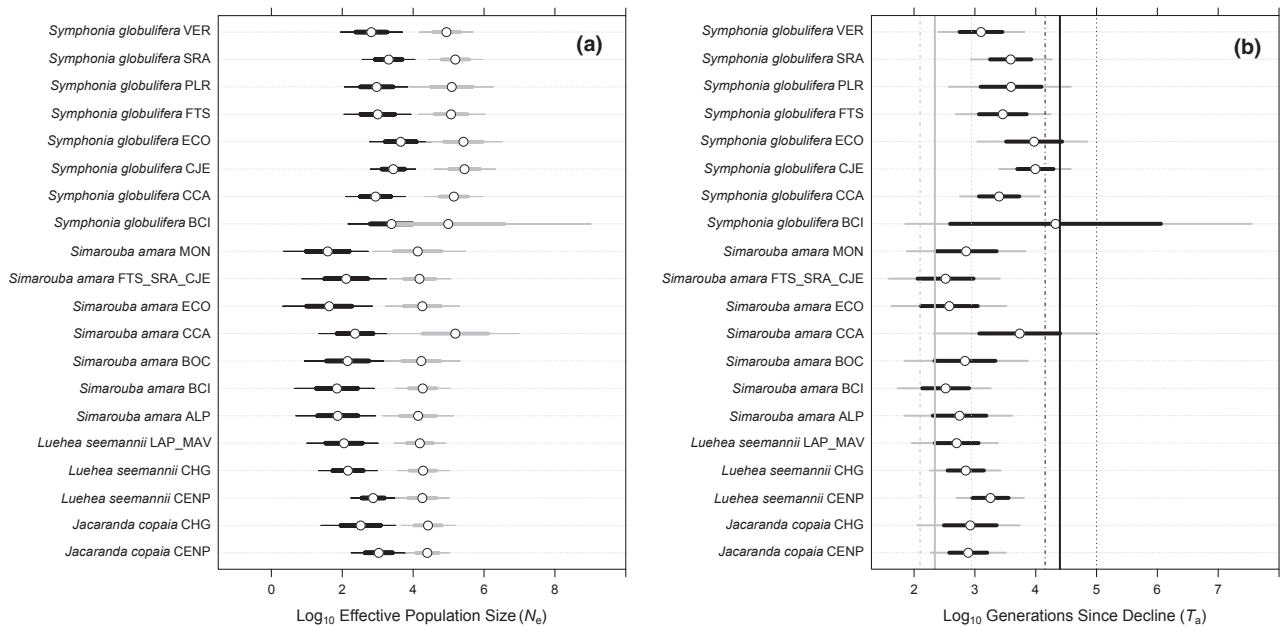
Figure 3 Results of the msvar analysis of past demographic changes for each of the four Neotropical tree species included in the study. In both panels, large circles are mean estimates, thick lines are standard deviations, and thinner lines are the 95% highest posterior density. (a) The ratio of current effective population sizes N_0 to ancient effective population sizes N_1 for all demes. (b) Number of generations since demographic change. The grey and black vertical lines represents the end of the Last Glacial Maximum (22 ka) and the end of the Pliocene (2500 ka) in tree generations, respectively. Slash-dotted, solid and dotted lines estimating these times assuming 25-, 100- and 175-year tree generation times, respectively. Sampling location codes are provided in Appendix S1.

mates for *L. seemannii* ranged from 25 ka (25 g_a) to 176 ka (175 g_a). Estimates for *S. amara* were 46 ka (25 g_a) and 326 ka (175 g_a). With the outlying CCA population removed, mean estimates for *S. amara* were 8.4 ka (25 g_a) to 113 ka (175 g_a). We acknowledge the broad overlap among demes within and among species in absolute and relative timing, as well as wide uncertainty intervals on these estimates that can span several orders of magnitude and produce very long times to coalescence that are unrealistic. The reasons for this are discussed below.

DISCUSSION

In the absence of a more complete recent fossil record, population genetic data can be used to reconstruct demographic histories and relate them to models of past environmental change (Pruett *et al.*, 2010; Girod *et al.*, 2011). Our comparative analysis found contrasting patterns of genetic structure in animal-dispersed evergreen trees and wind-dispersed seasonally deciduous trees. We also found genetic evidence of historical bottlenecks in each sampled deme regardless of the ecological differences among the study species. The demographic changes were coeval in three species that all happen to be light-demanding pioneer species, and their timing estimates broadly overlapped with the LGM, while only one species (*Symphonia globulifera*) showed evidence of demographic changes during an earlier time frame.

Population structure

We found substantial differences in genetic structure between the wind-dispersed *J. copaia* and *L. seemannii* and the animal-dispersed *Simarouba amara* and *Symphonia globulifera* in F_{ST} and the Bayesian clustering analyses. *Jacaranda copaia* and *L. seemannii* had lower F_{ST} and spatially extensive demes that spanned the Panama Canal watershed, a result consistent with high levels of gene flow. In contrast, we found stronger genetic structure for *Simarouba amara* and *Symphonia globulifera* with higher F_{ST} and a greater number of demes than the other species, consistent with more restricted gene flow.

These differences in genetic structure are likely to be influenced by differences in colonization history in combination with life-history traits that affect rates of colonization and gene movement. The best life-history predictor of genetic structure observed among plant species is the mating system (Hamrick & Godt, 1996; Duminil *et al.*, 2007), although the seed dispersal mechanism can also be also important (Hamrick & Godt, 1996; Nybom, 2004). Like many tropical forest trees (Bawa *et al.*, 1985; Dick *et al.*, 2008), the focal species here have been recorded as being primarily (80–100%) outcrossed due to self-incompatability (*J. copaia*, *S. globulifera*) or dioecy (*Simarouba amara*). Only *L. seemannii* is thought to have a mixed mating system with potential high levels of self-fertilization.

In our study, the highest inbreeding coefficients (F_{IS}) were observed in *S. amara* and *Symphonia globulifera*. The higher F_{IS} in *Simarouba amara* was unexpected because it is

a dioecious species, and it may be explained in part by the occurrence of null alleles. However, it is also worth noting that clumped kin-structured seed dispersal by animals combined with biparental inbreeding could also yield positive inbreeding coefficients in dioecious species (Hardesty et al., 2005; Yu et al., 2010). The generally lower structure (F_{IS} and F_{ST}) observed in the wind-dispersed species may be due to more efficient dispersal across a heterogeneous landscape. For example, direct genetic studies have confirmed the long-distance seed dispersal potential of J. copaia (Jones et al., 2005; Jones & Muller-Landau, 2008) and studies of fine-scale spatial genetic structure indicate higher levels of gene dispersal in J. copaia than in Symphonia globulifera (Hardy et al., 2006). Mechanistic models of dispersal in J. copaia and L. seemannii have also demonstrated the long-distance seed dispersal potential of these species through seed uplift (Wright et al., 2008). Finally, because these species show a greater tolerance of aridity than either S. globulifera or Simarouba amara due to their deciduous life history, they may have a broader range of habitats available for establishment and consequent population connectivity within Panama's past and present heterogeneous landscape. However, we note that J. copaia is constrained to wetter habitats that L. seemannii.

Demographic histories

Our results provide evidence for population bottlenecks in all four species at each deme examined (Table 4, Appendix S2). Population bottlenecks can arise from founding events or population reductions. False bottleneck signals can arise from cryptic genetic structure within sampled locations, but given that we did the analysis for multiple demes within each species, it is unlikely that all demes have cryptic population structure. Our results for J. copaia, L. seemannii and Simarouba amara suggest that current populations are either the result of founding events that coincided with the LGM, or that these species were persistent within the landscape at higher population densities and underwent population decline during this time period. MSVAR cannot differentiate between these two scenarios. Consistent with the population decline model is the hypothesis that the Caribbean slope once served as a refuge with sufficient precipitation and greater land area under lowered sea levels (Poelchau & Hamrick, 2013). It follows that the current large demes across this area (CENP for J. copaia and L. seemannii and the FTS/SRA/CJE deme for S. amara) could be remnants of larger demes that inhabited the now submerged Caribbean shelf (Fig. 1). These three species are also light-demanding pioneers that might have been able to rapidly colonize newly suitable areas from refuges. We acknowledge that the mean posterior estimates for ancient population sizes in some cases are much larger than current population sizes and the uncertainty in these estimates is shown in Fig. 3a. Similar scales of population bottlenecks (95 to > 99% reductions) have been detected using MSVAR in other tree species (Nettel et al., 2009).

The mechanisms by which Symphonia globulifera (and species with similar histories) may have persisted through the LGM merit consideration. While montane areas often maintain high levels of precipitation, Bush & Colinvaux (1990) and Bush et al. (1992), based on fossil pollen data, argued that mid-elevation montane areas probably did not serve as refuges for lowland tropical tree species during the LGM. Our results do not support an expansion out of refugial montane areas as we found no evidence of population expansion in any of our sampled upland locations; however some species were completely absent from these areas. But we did find longer time to coalescence at some high-elevation sites (e.g. population CCA for Simarouba amara). Persistence in upper-elevation sites is also supported by chloroplast DNA (cpDNA) sequence data for Symphonia globulifera (Dick & Heuertz, 2008) in which all upper-elevation sites in Panama were fixed for different haplotypes, suggesting long-term persistence and genetic isolation in these areas. Fossil pollen records indicate that the S. globulifera has been present in Central America at least since the Pliocene (Dick & Heuertz, 2008), which is consistent with our estimates of demographic change potentially as far back as the end of the Pliocene (Fig. 3b).

An alternative to the montane refuge hypothesis for S. globulifera is that mesic riverine habitats permitted wet-adapted species to persist in an overall drier regional landscape than currently exists (Piperno et al., 1990; Aide & Rivera, 1998). Meave & Kellman (1994) found that even isolated fragments of riverine forests could support a high diversity of common lowland species in a savanna matrix in Belize. In the case of S. globulifera, it is positively associated with seasonally flooded habitats and swamps in French Guiana and Africa where it can be monodominant, but it is also tolerant of experimental drought conditions at the seedling stage (Baraloto et al., 2007). The persistence of S. globulifera in the landscape before the LGM, as well as the high level of genetic differentiation seen among all populations of the species sampled, suggests that the species could have persisted in the landscape in isolated riverine forests or freshwater swamps while surrounding areas were composed of vegetation adapted to drier conditions.

One potential explanation for large uncertainty in some demes of S. globulifera is secondary contact between divergent populations. This would be expected to increase estimates of current and ancient effective population sizes as well as time to coalescence. In support of this scenario, Dick & Heuertz (2008) found two divergent cpDNA trnH–psbA haplotypes in central Panama indicative of contact with Caribbean slope populations.

It is worth noting the difficulty in mapping generation times and absolute times in our analysis. MSVAR assumes non-overlapping generations, and it is not clear what the most appropriate definition is for generation time in long-lived trees. We assume here that it is the average age at which a tree produces a successful offspring (i.e. an offspring that reaches adult stature and reproduces). In this case, the

average age is not the minimum age to reproduction (as used in many ecological studies) nor is it the mean age of maximal seed production. The true generation time cannot be known, but might be estimated from data on age structure and fecundity, which to our knowledge are not available for any long-lived tropical tree due to the absence of annual growth rings, the difficulty of assessing fecundity in closed forest, and the short time frame of most demographic studies. Our use of a broad range of generation times ranging from 25 to 175 years may overestimate the true generation time, although the older estimates are likely to encompass the true generation time of long-lived temperate forest trees (Clark & McLachlan, 2003; Petit & Hampe, 2006). Furthermore, Van Valen (1975) estimated the generation time of the palm *Euterpe globosa* to be approximately 100 years (with a maximum age of *c.* 175 years). Despite this uncertainty, all of our generation time estimates placed demographic changes in the Quaternary with the exception of some estimates for *S. globulifera*.

Our research points to the need to understand the complex assembly of local communities in the context of regional processes like dispersal and the non-equilibrial historical dynamics of individual species (Ricklefs, 2008). All of the focal species in our study coexist at different relative abundances in the central Panamanian locations sampled (FTS, BCI and PLR), but individual species may be absent or in low abundance from the other sampled locations either as a result of environmental tolerances or dispersal limitation. Our results demonstrate that when one considers the species that inhabit central Panama in a broader regional and historical context, species differ from one another in their levels of genetic structure within and among populations, effective population sizes, the extent of gene flow and dispersal among populations and, to some extent, in their demographic history. Furthermore, while these species are each distinct in their life histories, three of the four species, all pioneer trees, appear to have responded demographically in a similar way to Quaternary climate change. The implications of region-wide and historical dynamics on current demographic trends and predicted future dynamics of these species in response to future climate change remains to be explored.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Species descriptions and sampling locations.
Appendix S2 Pairwise F_{ST} and R_{ST} for each of the four study species.
Appendix S3 Posterior estimates for the MSVAR analysis.

BIOSKETCH

Andy Jones is an assistant professor in the Department of Botany and Plant Pathology, Oregon State University, and a research associate at the Smithsonian Tropical Research Institute. All the authors share research interests in the evolutionary ecology, population genetics, and historical biogeography of tropical trees.

Author contributions: F.A.J. and C.W.D. conceived the ideas; F.A.J., B.D.H. and C.W.D. collected data; I.C.S. and F.A.J. analysed data; F.A.J. and C.W.D. led writing of the paper. All authors commented on and discussed the paper.

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