Non-timber Forest Product Harvest does not Affect the Genetic Diversity of a Tropical Tree Despite Negative Effects on Population Fitness

Orou G. Gaoue[1,2,6], **Maristerra R. Lemes**[3,4], **Tamara Ticktin**[1], **Brice Sinsin**[2], and **Oscar Eyog-Matig**[5]

[1] Department of Botany, University of Hawaii at Manoa, Honolulu, HI, 96822, U.S.A.

[2] Laboratoire d'Ecologie Appliquée, Université d'Abomey Calavi, Cotonou, Benin

[3] Laboratório de Genética e Biologia Reprodutiva de Plantas, Instituto Nacional de Pesquisas da Amazônia, Manaus-AM, Brazil

[4] Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Jardim Botânico, Rio de Janeiro, RJ, Brazil

[5] Bioversity International, c/o CIFOR Regional Office, Yaounde, Cameroon

ABSTRACT

The level of genetic diversity in a population can affect ecological processes and plant responses to disturbance. In turn, disturbance can alter population genetic diversity and structure. Populations in fragmented and logged habitats often show reduced genetic diversity and increased inbreeding and differentiation. Long-term harvesting of wild plants (for foliage, bark, and roots), can affect population genetic diversity by altering individual fitness and genetic contribution. Our understanding of these changes in genetic diversity due to the harvesting of plant organs is still limited. We used nine microsatellite markers to study the effect of long-term bark and foliage harvest by Fulani people on the genetic diversity and structure of 12 populations of African mahogany (*Khaya senegalensis*) in Benin. We sampled 20 individuals in each population to test the effect of harvesting. For each population, we divided the samples equally between seedling and adults to test if the effects are stronger in seedlings. We found moderate genetic diversity ($H_e = 0.53 \pm 0.04$) and weak but significant differentiation among local populations ($F_{ST} = 0.043$, $P < 0.001$). There was no significant effect of harvest on genetic diversity or structure, although previous work found significant negative effects of harvest on the reproduction of adults, offspring density, and population fitness. Our results suggest that demographic responses to disturbance precede a detectable genetic response. Future studies should focus on using parentage analysis to test if genotypes of harvested parents are directly represented in the offspring populations.

Key words: bark harvest; disturbance; foliage harvest; genetic diversity; harvesting impact; *Khaya senegalensis*; microsatellites; tropical trees.

THERE IS A RECIPROCAL RELATIONSHIP BETWEEN THE GENETIC DIVERSITY OF POPULATIONS and their response to disturbance. The level of genetic diversity has critical effects on ecological processes and mitigates plant population responses to disturbance (Endels *et al.* 2007, Hughes *et al.* 2007, 2008). In turn, disturbance at the individual and population levels can alter genetic diversity and structure (Young *et al.* 1996, Pither *et al.* 2003). Disturbance can alter the proportion of polymorphic loci (Oostermeijer *et al.* 2003, Lowe *et al.* 2005), inbreeding coefficient (Andre *et al.* 2008), allele richness, and heterozygosity (Buchert *et al.* 1997). In a few cases, genetic diversity positively correlates with population fitness (see Oostermeijer *et al.* 2003, Reed & Frankham 2003, Finger *et al.* 2011) and community diversity (Booth & Grime 2003, Vellend 2006). Understanding if and how disturbance affects genetic diversity within and among populations can provide insights on adaptive capabilities within populations, potential for conservation, and risks of extinction.

Research in conservation genetics over the last two decades has focused on the genetic impacts of disturbance such as forest fragmentation (Young *et al.* 1996, Born *et al.* 2008), timber logging (Degen *et al.* 2006, Andre *et al.* 2008), and

domestication in traditional agroforestry parklands (Hollingsworth *et al.* 2005, Kyndt *et al.* 2009). For most of these disturbances, individual plants are killed and their genetic profile is removed from the population, as are their potential contributions to the gene pools. In contrast, disturbance such as harvesting of wild plants for non-timber forest product (*e.g.*, foliage, bark, fruits, flowers) does not usually directly remove individuals from the population, but rather alters their survival, growth, and reproduction.

Collection of non-timber forest products (NTFPs) is widespread and common across the tropics and is increasing in economic and socio-cultural significance (Bawa *et al.* 2004). NTFP harvesting often involves removal of reproductive structures such as fruits or seeds and may directly reduce the size of reproducing parent population or 'effective population size' (Namkoong *et al.* 1996). Overexploitation, even of non-reproductive parts can significantly affect individual physiology (Snyder & Williams 2003, Gaoue *et al.* 2011b), growth, survival, and population dynamics (Ticktin 2004). A few studies have also reported that harvesting can have a negative impact on the genetic diversity and structure of harvested populations (Cruse-Sanders *et al.* 2005, Mooney 2007). An improved understanding of how NTFP harvesting alters genetic diversity and structure can be used in conjunction with demographic data to determine sustainable harvesting limits

and to identify genetically unique populations worthy of greater conservation attention.

If harvesting can reduce the number of reproductive individuals and the number and quality of propagules contributing to future cohorts, it can act as a selective force and reduce population genetic diversity, specifically in individuals that established post-disturbance. It is expected that any effect of NTFP harvest on population genetic diversity and differentiation is more likely to be identified in the life stages (e.g., seedling and saplings) that established after disturbance (Cloutier et al. 2007, Kettle et al. 2007). Differences in gene diversity between juveniles (e.g., seedling and sapling) and adults can reveal potential temporal genetic erosion in environments where the viability of adults is low; this can be an early warning of chronic effect of disturbance on population genetic diversity (Kettle et al. 2007).

Khaya senegalensis (Desr.) A. Juss (Meliaceae) is an important mahogany tree in Africa that has been heavily harvested by local people for its bark, foliage, and timber over many decades. Khaya senegalensis is also one of the best timber trees in Africa and has been harvested for export since the 19th century (Normand & Sallenave 1958); in many places the species has been locally extirpated due to logging. The leaves, fruits and bark of K. senegalensis contain a wide range of biological compounds with anti-feedant, repellent, anti-inflammatory, and anti-microbial properties (Nakatani et al. 2000) that give the species high-commercial value for the agrochemical and pharmaceutical industries (Muellner et al. 2003). The stem bark is also used as traditional medicine for several human and livestock diseases (Atawodi et al. 2002). Less than 25 percent of trunk is often debarked with no direct lethal effect to the tree. However, there are rare cases of ring-barking which lead to trees death (Gaoue & Ticktin 2007).

The foliage of K. senegalensis is also harvested by the Fulani as dry season fodder for their livestock. Harvested trees often lose more than 80 percent of their canopy (Gaoue & Ticktin 2007) and most of harvested populations have been pruned since the drought of the 1970s. Cattle are a vital source of income and an integral part of the Fulani's culture. Bark and foliage harvest has been practiced over many decades. As a consequence, many individuals fail to reproduce (Gaoue & Ticktin 2008) and this could have reduced the contribution of non-reproductive mature individuals to the genetic pool of harvested populations, thereby reducing genetic diversity and altering populations genetic structure.

In this study, we tested the effect of bark and foliage harvest on the genetic diversity and structure of K. senegalensis seedling and adult populations. We investigated if harvesting reduced allelic richness, genetic diversity, and increased population differentiation and how these results vary between climatic regions (dry vs. moist). Population harvesting intensities (high vs. low) were estimated based on the percentage of foliage harvested and the percentage of trunk bark removed. Populations that were considered as high harvested had more than half of their trees defoliated and low-harvested populations were those with less than 10 percent of trees defoliated. We hypothesized that harvesting effects will be detected in juvenile rather than in adult populations given

that juveniles (less than 5-yr-old) have established recently (K. senegalensis does not have persistent seed bank). We discuss the implications of our results for conservation and for the genetic response of plant populations to disturbance. Although timber harvesting has a significantly more direct impact on population density and potentially on the genetic diversity, we knowingly chose to test the genetic impacts of non-timber forest product harvest particularly because this is rarely tested.

METHODS

STUDY SYSTEM, SAMPLE COLLECTION, AND DNA EXTRACTION.—Khaya senegalensis grows in the wild throughout a wide range of habitats in tropical Africa, from 8° to 14°N (Normand & Sallenave 1958, CTFT 1988). It is a semi-deciduous, shade-intolerant, and slow-growing tree that may reach up to 30 m in height and 3 m in girth, with a short bole, dense crown, and leaves composed of 3–4 pairs of elliptic leaflets. Khaya senegalensis is monoecious, wind dispersed, with flat winged seeds, and small white hermaphroditic flowers that are insect pollinated (CTFT 1988). In our study areas, K. senegalensis reaches a maximum diameter at breast height (dbh) of 136.2 cm which would be the equivalent of 187 yr considering an average diameter increment of 0.73 cm/yr for most tree species in the region (see Sokpon & Biaou 2002).

We collected leaves from 237 individuals in twelve different populations of K. senegalensis selected randomly among 29 populations previously surveyed in Benin (6°–12°50′N and 1°–3°40′E). Twenty individuals were randomly sampled from each population. To test if the effect of harvest is stronger in the seedling population, these 20 individuals were equally divided among adults (dbh > 20 cm) and seedlings (diameter at base < 5 cm). The dispersal distance for the study species is about 100 m in open habitats and may even be far less in closed habitats such as the gallery forest where most of the populations were found. These gallery forests have narrow width (5–15 m). We sampled seedling within permanent plots (previously used for the demographic study; see Gaoue & Ticktin 2007) that were often 0.5–1 km long with a width that covers the entire gallery width. Seeds dispersed from parent trees located in these plots are likely to fall within the population. Therefore, we assume that seedlings within a given population are more likely to have their maternal parent in that population than outside of the populations. We sampled seedlings far from the edges of these plots to avoid including the seedlings from seeds dispersed from neighboring plants outside of our plots. The geographic distance between populations was 15 km to 300 km. Six populations were selected within each geographical region: Sudano-Guinean (7°30′–9°30′N) versus Sudanian (9°30′–12°N) (see Gaoue & Ticktin 2007 for detailed description of these regions). Three of the six populations in each region were harvested at a rate of more than 50 percent of trees harvested for more than 50 percent of their crown and more than 10 percent of their trunk. The three other populations in each region were low or not harvested (hereafter non-harvested populations). These populations are part of a long-term demographic and genetic study designed to investigate the impact of

non-timber forest product harvest; and are described elsewhere (Gaoue & Ticktin 2007, Gaoue *et al.* 2011a). Leaves were preserved in silica gel at $-20°C$ until DNA extraction. Genomic DNA was extracted from all leaves samples using the Qiagen DNEasy Kit (Qiagen, Valencia, California, U.S.A.) following manufacturer protocols. The genetic analysis was conducted at the Genetic and Reproductive Biology laboratory of the 'Instituto Nacional de Pesquisas da Amazônia (INPA)' in Manaus, Brazil.

MICROSATELLITES ANALYSIS.—We used nine microsatellite markers developed for *Swietenia macrophylla* and successfully transferred in *K. senegalensis* (Lemes *et al.* 2011) to analyze the genetic diversity and structure in *K. senegalensis*. PCR amplification was carried out in a final reaction volume of 13 μl containing 0.9 μM of each primer, 1 unit Taq DNA polymerase, 200 μM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM NH_4, 1.5 mM $MgCl_2$), BSA (Bovine Serum Albumin – 2.5 mg/mL), 7.5 ng of template DNA, ultrapure water, and 1.25 μM each forward and reverse primers. PCR amplifications were performed using the following program: an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, annealing temperature for each locus (52°C for sm07, sm18, sm36; 56°C for sm01, sm08, sm12, sm46; 60°C for sm05, and 62°C for sm22) for 1 min and 72°C for 1 min; and a final elongation step at 72°C for 7 min. The PCR products were visualized in 2 percent agarose gels containing 0.1 μg/mL of ethidium bromide in 1X TBE buffer (89 mM Tris-borate, 2 mM EDTA pH 8.3) and sized with a 1Kb DNA ladder (Gibco, MD). Amplified products were resolved in 5 percent PAGE in an ABI 377XL sequencer and we estimated allele size for each sample and each locus using GENSCAN and GENOTYPER.

DATA ANALYSIS.—For each locus, each population, group of populations (harvested vs. non-harvested populations or moist vs. dry regions), and life stage (adults vs. seedlings), we estimated and compared the mean number of alleles per locus, A, allelic richness, R_s, a measure of the number of alleles independent of sample size (Petit *et al.* 1998), the expected H_e and observed H_o heterozygosity, Weir and Cockerham (1984)and population differentiation statistics (F_{IS}, F_{IT} and F_{ST}) in FSTAT 2.9.3.2 (Goudet 1995). For each of these parameters, we tested for significant deviance from zero using 1000 permutations. For F_{ST}, we used log-likelihood G-statistic (Goudet *et al.* 1996). We tested for significant differences in F_{IS}, F_{IT}, and F_{ST} between seedling and adult populations by comparing their 95 percent bootstrap confidence intervals. We tested each locus for deviations from Hardy–Weinberg expectations in each population using Wright's fixation index (F_{IS}) and tested for linkage disequilibrium in ARLEQUIN 3.11.

We tested for the effect of NTFP harvest on the genetic diversity in two separate ways. We first compared mean values of A, R_s, H_o, and H_e between groups (harvested vs. non-harvested regardless of climatic region, and harvested vs. non-harvested in each climatic region) without separating populations according to life stages ($N = 20$ per population) using 1000 permutations to determine the *P*-values in FSTAT 2.9.3.2. Second, to test if there was a stronger effect of harvest at the seedling level, we repeated the same tests, separating each population by life stages. This reduced our unit sample size to ten and may have limited the power to detect significant effect of harvest for this part of the analysis in spite of the resampling-based inference used by FSTAT 2.9.3.2. To rule out the hypothesis of isolation by geographic distance, we performed a Mantel test in ARLEQUIN 3.11 (Excoffier *et al.* 2005, updated from version 3.0). between log-transformed pairwise geographical distance between populations and their pairwise $F_{ST}/(1-F_{ST})$ (Rousset & Raymond 1997). Statistical significance for the correlation coefficient was determined using 1000 permutations. We performed an analysis of molecular variance (AMOVA) in ARLEQUIN 3.11 to test if there is a partitioning of genetic variance along the gradient of region by NTFP harvesting intensity. To test if NTFP harvest led to recent reduction in population effective size, we tested for significant excess in expected heterozygosity under the assumption that all loci fit the Infinite Allele Model (IAM) and in mutation-drift equilibrium, using a Wilcoxon sign-rank test with 1000 runs in BOTTLENECK 1.2.02 (Cornuet & Luikart 1996).

RESULTS

GENETIC DIVERSITY AND POPULATION DIFFERENTIATION.—The mean number of alleles per locus A across populations was 4.83 (SD = 0.32) and the allelic richness R_S was 3.82 (SD = 0.21) (Table S1). The observed heterozygosity H_o was 0.54 ± 0.06 and expected heterozygosity H_e across population was 0.53 ± 0.04. There was no significant difference in allelic richness ($P = 0.613$), observed ($P = 0.308$), and expected heterozygosities ($P = 0.583$) between adults and seedlings. This suggests no clear evidence of future genetic erosion.

Over all the nine loci, the inbreeding coefficients within individuals ($F_{IT} = 0.030$; 95% CI = $[-0.18; 0.20]$) and among individuals within each population ($F_{IS} = -0.01$; 95% CI = $[-0.22; 0.16]$) did not differ significantly from zero (Table 1). However, there was a weak but significant differentiation between populations ($F_{ST} = 0.043$; 95% CI = $[0.031; 0.059]$). Fifty-five percent of the pairwise population differentiation coefficients were significantly different from zero (Table S2). Sinisson and Okpara populations, both located in the Sudano-Guinean region, were the most differentiated from the other populations. The highest pairwise F_{ST} values (=0.109, $P < 0.001$) were found between Dogue and Gbeba populations (Table S2). Both populations were heavily harvested and located in different regions. However, the Mantel test did not support the hypothesis of isolation by geographic distance ($r = -0.148$, $P = 0.865$).

IMPACT OF HARVEST ON GENETIC DIVERSITY AND STRUCTURE.—There was no significant difference in the allelic richness ($P = 0.589$), observed ($P = 0.161$) and expected heterozygosities ($P = 0.434$) between harvested and non-harvested populations within each climatic region. We also did not find significant differences in allelic richness ($P = 0.317$), observed ($P = 0.443$), and expected

TABLE 1. *Inbreeding and genetic differentiation estimates (mean ± 1 SE) for each of nine loci, the overall mean over the nine loci[a], and for each life stage (seedlings and adults)[b].* F_{IS}: *within population inbreeding coefficient;* F_{IT}: *overall inbreeding coefficient;* F_{ST}: *estimates of Wright's fixation index* F_{ST} *(Weir & Cockerham 1984). P-value significance were determined from 1000 randomization log-likelihood G (Goudet* et al. *1996). The confidence interval (CI) limits were obtained from 1000 bootstraps runs[c].*

Locus	F_{IS}	F_{IT}	F_{ST}
sm01	0.203 ± 0.058	0.241 ± 0.052	0.047 ± 0.018
sm46	0.040 ± 0.059	0.167 ± 0.06	0.132 ± 0.030
sm05	0.104 ± 0.057	0.141 ± 0.055	0.041 ± 0.015
sm07	-0.755 ± 0.037	-0.752 ± 0.037	0.002 ± 0.003
sm22	0.255 ± 0.075	0.278 ± 0.068	0.031 ± 0.024
sm12	0.324 ± 0.089	0.366 ± 0.079	0.063 ± 0.030
sm18	0.081 ± 0.054	0.114 ± 0.055	0.036 ± 0.019
sm08	-0.017 ± 0.036	0.025 ± 0.032	0.041 ± 0.017
sm36	-0.145 ± 0.081	-0.084 ± 0.072	0.053 ± 0.014
Overall mean[a] [95% CI[c]]	-0.014^{ns} [-0.224; 0.155]	0.030^{ns} [-0.184; 0.198]	0.043^{**} [0.031; 0.059]
Seedlings[b] [95% CI]	-0.009^{ns} [-0.227; 0.170]	0.038^{ns} [-0.181; 0.216]	0.046^{**} [0.030; 0.070]
Adults[b] [95% CI]	-0.031^{ns} [-0.237; 0.135]	0.022^{ns} [-0.189; 0.186]	0.052^{**} [0.034; 0.072]

$^{**}P < 0.01$; $^{*}P < 0.05$; ns, non significant.

heterozygosities ($P = 0.112$) between harvested and non-harvested populations regardless of region. These results indicate a lack of clear effect of NTFP harvest on *K. senegalensis* genetic diversity at the whole population level. Similarly, there was no significant effect of harvest on these genetic parameters for seedlings or adults when we analyzed the data separately.

We found a weak but marginally significant genetic structure related to climatic region and harvesting intensity (0.80 percent of variation; $F_{CT} = 0.008$, $P = 0.042$). The analysis of molecular variance for seedlings did not reveal any population structuring related to region or harvesting intensity. Genetic variation in seedlings was mainly due to strong differences among individuals (89 percent of variation; $F_{IT} = 0.111$, $P < 0.0001$). There was less genetic variation among individuals nested in populations (6 percent of variation; $F_{IS} = 0.064$, $P < 0.011$) and among populations within each combination of region by harvest intensity (5 percent; $F_{SC} = 0.050$, $P < 0.0001$). Nine percent of loci pairs were linked.

Under the infinite allele model (IAM), nearly 83 percent of the populations we surveyed experienced a recent significant or marginally significant bottleneck (Wilcoxon Sign test; 1000 runs in BOTTLENECK 1.2.02; $P < 0.05$; Table 2). Four out of six populations that revealed significant recent bottlenecks under different models (IAM, TPM, and SMM) were harvested. Dogue (high-harvested population in the Sudano-Guinean region) and Barabon (non-harvested population in the Sudanian region) revealed marginally significant bottleneck under IAM but no significant reduction in their effective population size under SMM or TPM models. Populations that did not show consistent significant evidence of recent reduction in their effective population size (Penelan, Nipuni, Barabon) were non-harvested for NTFP and for timber over the past years. However, two low-harvest populations showed significant recent bottleneck (Fetekou: $P = 0.004$; Sinisson: $P < 0.010$) and this may suggest a compounding effect of past logging.

TABLE 2. *Probability of excess of heterozygotes for 12 populations of* Khaya senegalensis *under three models of mutation: infinite allele model (IAM), two-phase model TPM (with variance = 30 and probability = 70 percent), and stepwise mutation model (SMM). P-values are for one-sided Wilcoxon tests after 1000 replicates to detect significant recent bottlenecks for each population (Cornuet & Luikart 1996).*

Ecological region and Harvest intensity	Population	IAM	TPM	SMM
Sudano-Guinean Low harvest	Boukoussera	0.042^{*}	0.312^{ns}	0.312^{ns}
	Sinisson	0.010^{**}	0.019^{*}	0.019^{*}
	Penelan	0.080^{ns}	0.215^{ns}	0.215^{ns}
Sudano-Guinean High harvest	Dogue	0.052^{*}	0.161^{ns}	0.216^{ns}
	Okpara	0.004^{**}	0.010^{**}	0.014^{*}
	Sakarou	0.014^{*}	0.125^{ns}	0.150^{ns}
Sudanian Low harvest	Nipuni	0.082^{ns}	0.179^{ns}	0.212^{ns}
	Barabon	0.053^{ns}	0.116^{ns}	0.116^{ns}
	Fetekou	0.004^{**}	0.010^{**}	0.014^{*}
Sudanian High harvest	Gbeba	0.002^{**}	0.002^{**}	0.002^{**}
	Nigoussourou	0.007^{**}	0.012^{*}	0.065^{ns}
	Soassararou	0.004^{**}	0.006^{**}	0.010^{**}

$^{**}P < 0.01$; $^{*}P < 0.05$; ns, non significant.

DISCUSSION

Our study showed that *Khaya senegalensis* populations have moderate genetic diversity ($H_e = 0.53 \pm 0.04$) and weak but significant population differentiation ($F_{ST} = 0.043$, $P < 0.001$). The genetic diversity in our study is lower than reported for other mahogany species (*e.g.*, Lemes *et al.* 2003, Cloutier *et al.* 2007, Ismail *et al.* 2012). In contrast to previously reported effects of disturbance such as forest fragmentation on genetic diversity (Leimu *et al.*

2006, Honnay & Jacquemyn 2007, Aguilar *et al.* 2008), there was no negative effect of harvest on *K. senegalensis* genetic diversity regardless of life stage, even though previous work found significant negative effects of harvest on the reproductive contribution of adults (Gaoue & Ticktin 2008: Fig. 2), seedling recruitment (Gaoue & Ticktin 2007) and on populations fitness (Gaoue *et al.* 2011a).

We also found that populations with higher levels of NTFP harvesting produced significantly less fruits and seeds per tree with a significantly lower proportion of fruiting trees than low-harvest populations (Gaoue & Ticktin 2008). This has translated into significantly lower seedlings density in harvested populations (Gaoue & Ticktin 2007). Similarly, several other studies on tropical trees also report that disturbance such as logging (see Cloutier *et al.* 2007, Kettle *et al.* 2007, Silva *et al.* 2008) or NTFP harvesting in China (Wang *et al.* 2013, Xu *et al.* 2013) may not always lead to a detectable reduction in genetic diversity. However, our finding does not support those of Cruse-Sanders *et al.* (2005) and Mooney (2007), who documented reduced genetic diversity in root-harvested populations of American ginseng.

The lack of significant effect of NTFP harvesting on the genetic diversity is likely due to several factors related to the nature of the NTFP harvest, the biology of the study species, the nature of the markers used, the compounding effect of logging in some of our populations, and the low sample sizes used in this study. While root harvest (*e.g.*, American ginseng) often kills individuals, harvesting foliage and bark may reduce reproductive outputs and decrease the representation of various genotypes in the progeny but is not strong enough to keep some genotypes out of the gene pool. We did not find any genetic effect of harvesting, even in the seedlings that were established after the heavy foliage harvesting that has taken place since the 1970s. This suggests no clear future genetic erosion as a consequence of bark and foliage harvesting. That is, the nearly 40 yr during which harvest intensity has increased significantly was not sufficient to yield detectable genetic erosion in the seedling populations. The mechanisms by which disturbance could alter genetic diversity include a reduction in the fitness of disturbed individuals and populations, an increase in the distance between individuals and limited gene flow due to low pollen exchange or seed dispersal. The lack of reduction in diversity in the seedling populations could be related to the arrival of seeds dispersed from neighboring populations with similar genetic profiles. This is consistent with the low genetic differentiation we found. *Khaya senegalensis* is wind dispersed and in other mahogany such as *Swietenia humilis*, long-distance pollen and seed dispersal has been reported (White *et al.* 2002). Therefore, dispersal may compensate for the reduction in genetic diversity caused by disturbance such as fragmentation or heavy harvesting (*e.g.*, Born *et al.* 2008).

In spite of the widespread use of microsatellites in conservation genetics, as neutral markers they may underestimate total genetic diversity (Vǎli *et al.* 2008). The neutral genetic diversity reveal by these markers are a poor surrogate of the adaptive genetic diversity (Holderegger *et al.* 2006) that are more sensitive to environmental changes such as the one exert by harvesting. This lack of perfect correlation between neutral and adaptive genetic diversity (Reed & Frankham 2003) may also explain the lack of correlation between *K. senegalensis* genetic diversity, fruits and seeds production, density and population fitness.

Genetic diversity in *Khaya senegalensis* populations was similar to that of other wild tree species in Africa (Ouinsavi *et al.* 2006, Born *et al.* 2008). However, even *K. senegalensis* populations that were harvested had higher genetic diversity than reported for agroforestry trees (*Adansonia digitata* and *Vitellaria paradoxa*) that have been heavily harvested for fruits and seeds over decades (Allaye Kelly *et al.* 2004, Sanou *et al.* 2005, Assogbadjo *et al.* 2006, Kyndt *et al.* 2009). One of the main differences between *K. senegalensis* and these agroforestry species is the type of organ they are harvested for. The relatively higher genetic diversity in our study suggests that the genetic impact of NTFP harvesting depends upon the type of organs harvested. Although harvesting fruits may directly affect the genetic diversity of progeny, this is not necessarily the case for the harvest of foliage and bark. Another explanation for the relatively low genetic diversity in agroforestry species may be the low density of trees in these populations (*e.g.*, five stems/ha for *A. digitata*; Kyndt *et al.* 2009; 8–20 stems/ha for *V. paradoxa*; Djossa *et al.* 2008) compared to *K. senegalensis* which density was 43.16 ± 7.23 stems/ha (38.16 ± 8 trees/ha in harvested populations) in our study areas (Gaoue & Ticktin 2007). There is a global positive relationship between population size and genetic diversity and fitness (Young *et al.* 1996, Aguilar *et al.* 2008). The low density of trees in agroforestry species may reduce the probability of outcrossing.

Our study is one of very few to investigate the genetic impacts of NTFP harvest (see Cruse-Sanders & Hamrick 2004, Mooney 2007) and the genetic diversity and structure of trees in tropical Africa (Allaye Kelly *et al.* 2004, Dick *et al.* 2007, Kyndt *et al.* 2009). Although we did not detect a significant effect of harvest on the genetic diversity, our study provides a useful contribution to our understanding of the genetic consequences of NTFP harvesting. Nevertheless, we found recent bottlenecks in many harvested populations. This may be due to concurrent timber harvest that had taken place in these populations. Except for a few cases in the National Park of 'W' (Barabon and Nipuni populations), populations in our study system have been logged at least once in their lifetime. Current rate of NTFP harvest from *K. senegalensis* may not affect its genetic diversity but it does reduce population fitness (Gaoue *et al.* 2011a) and may lead to gradual species decline before the negative effects of harvest on genetic diversity and structure could be detected. Future studies on the genetic impact of NTFP harvest should focus on using parentage analysis to test if genotypes of harvested parents are represented in the offspring populations.

ACKNOWLEDGMENTS

SUPPORTING INFORMATION

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

TABLE S1. *Mean number of alleles per locus, allelic richness, expected and observed heterozygosity and inbreeding coefficient for 12 populations of* Khaya senegalensis.

TABLE S2. *Pairwise genetic differentiation and geographic distance matrices between* Khaya senegalensis *populations.*

LITERATURE CITED

AGUILAR, R., M. QUESADA, L. ASHWORTH, Y. HERRERIAS-DIEGO, AND J. LOBO. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17: 5177–5188.

ALLAYE KELLY, B., O. HARDY, AND J.-M. BOUVET. 2004. Temporal and spatial genetic structure in *Vitellaria paradoxa* (shea tree) in an agroforestry system in southern Mali. Mol. Ecol. 13: 1231–1240.

ANDRE, T., M. R. LEMES, J. GROGAN, AND R. GRIBEL. 2008. Post-logging loss of genetic diversity in a mahogany (*Swietenia macrophylla* King, Meliaceae) population in Brazilian Amazonia. For. Ecol. Manage. 255: 340–345.

ASSOGBADJO, A. E., T. KYNDT, B. SINSIN, G. GHEYSEN, AND P. van DAMME. 2006. Patterns of genetic and morphometric diversity in baobab (*Adansonia digitata*) populations across different climatic zones of Benin (West Africa). Ann. Bot. 97: 819–830.

ATAWODI, S. E., D. A. AMEH, S. IBRAHIM, J. N. ANDREW, H. C. NZELIBE, E. O. ONYIKE, K. M. ANIGO, E. A. ABU, D. B. JAMES, G. C. NJOKU, AND A. B. SALLAU. 2002. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. J. Ethnopharmacol. 79: 279–282.

BAWA, K. S., J. W. KRESS, M. N. NADKARNI, AND S. LELE. 2004. Beyond paradise: Meeting the challenges in tropical biology in the 21st century. Biotropica 36: 437–446.

BOOTH, R. E., AND J. P. GRIME. 2003. Effects of genetic impoverishment on plant community diversity. J. Ecol. 91: 721–730.

BORN, C., O. J. HARDY, M.-H. CHEVALLIER, S. OSSARI, C. ATTEKE, E. J. WICKINGS, AND M. HOSSAERT-MCKEY. 2008. Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: A stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. Mol. Ecol. 17: 2041–2050.

BUCHERT, G. P., O. P. RAJORA, J. V. HOOD, AND B. P. DANCIK. 1997. Effects of harvesting on genetic diversity in old-growth Eastern white pine in Ontario, Canada. Conserv. Biol. 11: 747–758.

CLOUTIER, D., M. KANASHIRO, A. Y. CIAMPI, AND D. J. SCHOEN. 2007. Impact of selective logging on inbreeding and gene dispersal in an Amazonian tree population of *Carapa guianensis* Aubl. Mol. Ecol. 16: 797–809.

CORNUET, J. M., AND G. LUIKART. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

CRUSE-SANDERS, J. M., AND J. L. HAMRICK. 2004. Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (Araliaceae). Am. J. Bot. 91: 540–548.

CRUSE-SANDERS, J. M., J. L. HAMRICK, AND J. A. AHUMADA. 2005. Consequences of harvesting for genetic diversity in American ginseng (*Panax quinquefolius* L.): A simulation study. Biodivers. Conserv. 14: 493–504.

CTFT. 1988. *Khaya senegalensis* (Desr.) A. Juss. Bois et Forets des Tropiques 218: 43–56.

DEGEN, B., L. BLANC, H. CARON, L. MAGGIA, A. KREMER, AND S. GOURLET-FLEURY. 2006. Impact of selective logging on genetic composition and demographic structure of four tropical tree species. Biol. Conserv. 131: 386–401.

DICK, C. W., E. BERMINGHAM, M. R. LEMES, AND R. GRIBEL. 2007. Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. Mol. Ecol. 16: 3039–3049.

DJOSSA, B., J. FAHR, T. WIEGAND, B. AYIHOUÉNOU, E. KALKO, AND B. SINSIN. 2008. Land use impact on *Vitellaria paradoxa* C.F. Gaerten. stand structure and distribution patterns: a comparison of Biosphere Reserve of Pendjari in Atacora district in Benin. Agrofor. Syst. 72: 205–220.

ENDELS, P., H. JACQUEMYN, R. BRYS, AND M. HERMY. 2007. Genetic erosion explains deviation from demographic response to disturbance and year variation in relic populations of the perennial *Primula vulgaris*. J. Ecol. 95: 960–972.

EXCOFFIER, L., G. LAVAL, AND S. SCHNEIDER. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47–50.

FINGER, A., C. KETTLE, C. KAISER-BUNBURY, T. VALENTIN, D. DOUDEE, D. MATATIKEN, AND J. GHAZOUL. 2011. Back from the brink: Potential for genetic rescue in a critically endangered tree. Mol. Ecol. 20: 3773–3784.

GAOUE, O. G., C. C. HORVITZ, AND T. TICKTIN. 2011a. Non-timber forest product harvest in variable environments: modeling the effect of harvesting as a stochastic sequence. Ecol. Appl. 21: 1604–1616.

GAOUE, O. G., L. SACK, AND T. TICKTIN. 2011b. Human impacts on leaf economics in heterogeneous landscapes: The effect of harvesting non-timber forest products from African mahogany across habitats and climates. J. Appl. Ecol. 48: 844–852.

GAOUE, O. G., AND T. TICKTIN. 2007. Patterns of harvesting foliage and bark from the multipurpose tree *Khaya senegalensis* in Benin: Variation across ecological regions and its impacts on population structure. Biol. Conserv. 137: 424–436.

GAOUE, O. G., AND T. TICKTIN. 2008. Impacts of bark and foliage harvest on *Khaya senegalensis* (Meliaceae) reproductive performance in Benin. J. Appl. Ecol. 45: 34–40.

GOUDET, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. J. Hered. 86: 485–486.

GOUDET, J., M. RAYMOND, de-MEEUS T., AND F. ROUSSET. 1996. Testing differentiation in diploid populations. Genetics 144: 1933–1940.

HOLDEREGGER, R., U. KAMM, AND F. GUGERLI. 2006. Adaptive vs. neutral genetic diversity: implications for landscape genetics. Landscape Ecol. 21: 797–807.

HOLLINGSWORTH, P. M., I. K. DAWSON, W. P. GOODALL-COPESTAKE, J. E. RICHARDSON, J. C. WEBER, C. SOTELO MONTES, AND R. T. PENNINGTON. 2005. Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. Mol. Ecol. 14: 497–501.

HONNAY, O., AND H. JACQUEMYN. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conserv. Biol. 21: 823–831.

HUGHES, A. R., J. E. BYRNES, D. L. KIMBRO, AND J. J. STACHOWICZ. 2007. Reciprocal relationships and potential feedbacks between biodiversity and disturbance. Ecol. Lett. 10: 849–864.

HUGHES, A. R., B. D. INOUYE, M. T. J. JOHNSON, N. UNDERWOOD, AND M. VELLEND. 2008. Ecological consequences of genetic diversity. Ecol. Lett. 11: 609–623.

ISMAIL, S., J. GHAZOUL, G. RAVIKANTH, R. UMA SHAANKER, C. KUSHALAPPA, AND C. KETTLE. 2012. Does long-distance pollen dispersal preclude

inbreeding in tropical trees? Fragmentation genetics of Dysoxylum malabaricum in an agro-forest landscape. Mol. Ecol. 21: 5484–5496.

KETTLE, C. J., P. M. HOLLINGSWORTH, T. JAFFRE, B. MORAN, AND R. A. ENNOS. 2007. Identifying the early genetic consequences of habitat degradation in a highly threatened tropical conifer, *Araucaria nemorosa* Laubenfels. Mol. Ecol. 16: 3581–3591.

KYNDT, T., A. E. ASSOGBADJO, O. J. HARDY, R. GLELE KAKAI, B. SINSIN, P. VAN DAMME, AND G. GHEYSEN. 2009. Spatial genetic structuring of baobab (*Adansonia digitata*, Malvaceae) in the traditional agroforestry systems of West Africa. Am. J. Bot. 96: 950–957.

LEIMU, R., P. I. A. MUTIKAINEN, J. KORICHEVA, AND M. FISCHER. 2006. How general are positive relationships between plant population size, fitness and genetic variation? J. Ecol. 94: 942–952.

LEMES, M. R., T. B. ESASHIKA, AND O. G. GAOUE. 2011. Microsatellite markers for mahoganies: Twelve new loci for *Swietenia macrophylla* and its high transferability to *Khaya senegalensis*. Am. J. Bot. 98: e207–e209.

LEMES, M. R., R. GRIBEL, J. PROCTOR, AND D. GRATTAPAGLIA. 2003. Population genetic structure of mahogany (*Swietenia macrophylla* King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. Mol. Ecol. 12: 2875–2883.

LOWE, A. J., D. BOSHIER, M. WARD, C. F. E. BACLES, AND C. NAVARRO. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95: 255–273.

MOONEY, E. H. 2007. Genetic and evolutionary consequences of harvest in American ginseng, *Panax quinquefolius* L. (Araliaceae). PhD Dissertation. West Virginia University, Morgantown.

MUELLNER, A. N., R. SAMUEL, S. A. JOHNSON, M. CHEEK, T. D. PENNINGTON, AND M. W. CHASE. 2003. Molecular phylogenetics of Meliaceae (Sapindales) based on nuclear and plastid DNA sequences. Am. J. Bot. 90: 471–480.

NAKATANI, M., S. A. M. ABDELGALEIL, H. OKAMURA, T. IWAGAWA, A. SATO, AND M. DOE. 2000. Khayanolides A and B, new rearranged phragmalin limonoid antifeedants from *Khaya senegalensis*. Tetrahedron Lett. 41: 6473–6477.

NAMKOONG, G., T. BOYLE, H.-R. GREGORIUS, H. JOLY, O. SAVLAINEN, W. RATNAM, AND A. YOUNG. 1996. Testing criteria and indicators for assessing the sustainability of forest management: genetic criteria and indicators. CIFOR Working Paper, Jakartar.

NORMAND, D., AND P. SALLENAVE. 1958. Caracteristiques et proprietes des acadjous (*Swietenia* et *Khaya*). Bois et Forets des Tropiques 59: 43–52.

OOSTERMEIJER, J. G. B., S. H. LUIJTEN, AND J. C. M. DEN NIJS. 2003. Integrating demographic and genetic approaches in plant conservation. Biol. Conserv. 113: 389–398.

OUINSAVI, C., N. SOKPON, J. BOUSQUET, C. H. NEWTON, AND D. P. KHASA. 2006. Novel microsatellite DNA markers for the threatened African endemic tree species, *Milicia excelsa* (Moraceae), and cross-species amplification in *Milicia regia*. Mol. Ecol. Notes 6: 480–483.

PETIT, R. J., A. EL MOUSADIK, AND O. PONS. 1998. Identifying populations for conservation on the basis of genetic markers. Conserv. Biol. 12: 844–855.

PITHER, R., J. S. SHORE, AND M. KELLMAN. 2003. Genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in naturally fragmented populations. Heredity 91: 307–313.

REED, D. H., AND R. FRANKHAM. 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17: 230–237.

ROUSSET, F., AND M. RAYMOND. 1997. Statistical analyses of population genetic data: New tools, old concepts. Trends Ecol. Evol. 12: 313–317.

SANOU, H., P. N. LOVETT, AND J. M. BOUVET. 2005. Comparison of quantitative and molecular variation in agroforestry populations of the shea tree (*Vitellaria paradoxa* C.F. Gaertn) in Mali. Mol. Ecol. 14: 2601–2610.

SILVA, B. M., M. KANASHIRO, A. Y. CIAMPI, I. THOMPSON, AND A. M. SEBBENN. 2008. Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon. For. Ecol. Manage. 255: 1548–1558.

SNYDER, K. A., AND D. G. WILLIAMS. 2003. Defoliation alters water uptake by deep and shallow roots of *Prosopis velutina* (Velvet Mesquite). Funct. Ecol. 17: 363–374.

SOKPON, N., AND S. H. BIAOU. 2002. The use of diameter distributions in sustained-use management of remnant forests in Benin: case of Bassila forest reserve in North Benin. For. Ecol. Manage. 161: 13–25.

TICKTIN, T. 2004. The ecological implications of harvesting non-timber forest products. J. Appl. Ecol. 41: 11–21.

VÄLI, Ü., A. EINARSSON, L. WAITS, AND H. ELLEGREN. 2008. To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? Mol. Ecol. 17: 3808–3817.

VELLEND, M. 2006. The consequences of genetic diversity in competitive communities. Ecology 87: 304–311.

WANG, X.-Q., Y. HUANG, AND C.-L. LONG. 2013. Assessing the genetic consequences of flower-harvesting in *Rhododendron decorum* Franchet (Ericaceae) using microsatellite markers. Biochem. Syst. Ecol. 50: 296–303.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

WHITE, G. M., D. H. BOSHIER, AND W. POWELL. 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: An example from *Swietenia humilis* Zuccarini. Proc. Natl Acad. Sci. USA 99: 2038–2042.

XU, W., W. BAI, G. WEN, H. HUAI, AND A. LIU. 2013. Influences of harvesting on genetic diversity and population structure of *Anemone altaica* (Ranunculaceae), a traditional Chinese medicinal herb. Biochem. Syst. Ecol. 47: 121–125.

YOUNG, A., T. BOYLE, AND T. BROWN. 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11: 413–418.