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Spatial distribution of genetic diversity in populations of *Hagenia abyssinica* (Bruce) J.F. Gmel from Ethiopia

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Abstract. Genetic variation among 596 individuals from 22 natural and 3 planted populations of *Hagenia abyssinica* (Rosaceae) sampled from the montane forests of Ethiopia was investigated at amplified fragment length polymorphism (AFLP) loci. We observed 106 unequivocally scorable AFLP markers out of which 91.5 percent were polymorphic. Populations harbored varying genetic diversities (H_e = 0.139-0.362), and showed low but significant genetic differentiation among them (F_{ST} = 0.077). Significant differentiation was observed even though previous paleoecological studies indicated that *Hagenia abyssinica* recolonized Ethiopia only after the Last Glacial Maximum, and our earlier analyses of maternally inherited chloroplast DNA revealed low mixing of recolonizing lineages through seeds and rare long distance seed dispersal. Genetic diversity did not decrease along recolonization routes, confirming effective gene flow, most likely through pollen, among populations. The observed variation at putatively neutral AFLPs does not reflect clinal variation patterns. As expected, population differentiation is lower at anonymous, mostly biparentally inherited, AFLPs than at maternally inherited chloroplast haplotypes. Despite presumably efficient seed and pollen dispersal of *H. abyssinica* by wind, a significant non-random fine-scale spatial genetic structure was observed up to 80 m in some populations. Due to significant pair-wise differentiation observed between populations, as many populations as possible should be considered for conservation, tree improvement and forestation programs.
Keywords AFLPs, genetic diversity, kinship coefficient, population differentiation, recolonization, spatial genetic structure

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Introduction

Patterns of genetic diversity are affected by evolutionary processes and life history traits that collectively define the population's genetic structure (e.g., Yeh 2000). Tree species are generally characterized by high levels of genetic diversity within populations and relatively low levels of differentiation among populations (Loveless & Hamrick 1984, Finkeldey & Hattemer 2007, White et al. 2007). The geographic variation of genetic diversity has important implications for the ecological (e.g., adaptation to changing environmental conditions) and evolutionary (e.g., speciation) potential of populations (e.g., Hoffmann & Blows 1994) and for the conservation and use of genetic resources (e.g., Bawa & Krugman 1990). Genetic diversity is rarely distributed homogeneously within populations and genetic similarity is often higher among neighboring than among distant individuals (Vekemans & Hardy 2004; Jump & Peñuelas 2007). Such fine-scale genetic structure is affected by the dispersal ability, the mating system (higher in selfing species), life form (higher in herbs than trees) and population density (higher under low density) of a species (Vekemans & Hardy 2004, Cavers et al. 2005, Jump & Peñuelas 2007, Hardy et al. 2006).

Several studies on colonization history detected a decreasing genetic diversity with increasing distance from refugial sources based on contemporary patterns of genetic diversity (Rivera-Ocasio et al. 2002; Coart et al. 2005; Kebede et al. 2007). The decrease of genetic diversity during colonization is due to successive founder events and possibly further reduced by drift due to the small size of new populations (Wright 1969, Nei 1987, Rivera-Ocasio et al. 2002). Contrarily, other studies reported higher genetic diversity away from source populations due to gene flow and population admixture effects (e.g., Comps et al. 2001, Petit et al. 2003).

Hedberg (1989) and Negash (1995) reported that *Hagenia abyssinica* (Bruce) J.F. Gmel. is a predominantly wind-pollinated and wind-dispersed broad-leaved dioecious tree species belonging to a monotypic genus in the Rosaceae family. Nonetheless, the bright colourful and appealing appearance of the flowers of *H. abyssinica* (Supporting Information 2) is not typical for wind-pollinated species, suggesting that other pollinating vectors such as insects (particularly bees) might be involved in pollination. This view is supported by reports of honeybees collecting pollen from the male flowers and nectar from the female flowers (Fichtl & Admasu 1994). Diaspores are very light (weight of 1000 seeds: 2.2 g) and can easily be dispersed by wind. The species is found in 12 countries in Africa stretching from Ethiopia in the North to Zimbabwe in the South and inland to Congo (Hedberg 1989). It grows within an altitudinal range of 1,850 to 3,700 m asl (Hedeberg 1989, Friis 1992, Azene et al. 1993, Negash 1995) inhabiting the montane forests, montane woodlands and montane grasslands. The extant *Hagenia* populations throughout Ethiopia are situated at higher altitudes, often in wetter depressions (Ayele et al. 2009). Lange, Bussmann, & Beck (1997) reported that the regeneration capability of *H. abyssinica* was limited by herbivores. *Hagenia abyssinica* is a multipurpose tree species bestowed with considerable economic and ecological values. It is one of the best timber species in Ethiopia preferred for its strength, fine texture and attractive appearance. A concoction made from the powder of dried female inflorescences is used as a purgative and taenicide against tapeworm in Ethiopia (see Ayele *et al.* 2011 and the references therein). But due to over-exploitation, the species is gravely endangered in its natural range particularly in Ethiopia (Negash 1995) with only about 7000 individuals left in the wild (Ayele et al. 2011). Ayele et al. (2009) inferred that H. abyssinica recolonized Ethiopia in the late Pleistocene (since 16,700 years before present) from southern African countries based on evaluation of fossil pollen records (Beuning et al.

1997, Bonnefille et al. 1995, Olago et al. 1999, Umer et al. 2007). A recent phylogeographic investigation using maternally inherited chloroplast markers supported the recolonization hypothesis and suggested a single entry point into Ethiopia (Ayele et al. 2009). The recolonization route of the species was reconstructed, and rare mutation and long distance seed dispersal events were identified. A very high population differentiation (N_{ST} = 0.926) was reported whilst six haplotypes, which were grouped in two lineages, were identified (Ayele *et al.* 2009). The genetic diversity of few populations of *H. abyssinica* was investigated by RAPD (Kumilign 2005) and ISSR (Feyissa 2007) markers. Both studies covered a small spatial scale contrasting to the widespread natural distribution of the species in Ethiopia, and were also limited to small numbers of individuals per population.

In the present study, we cover the whole natural distribution range of *H. abyssinica* in Ethiopia to examine: 1) genetic diversity within and among populations, 2) changes in genetic diversity of the species along its recolonization routes, and 3) fine-scale spatial genetic structure. Based on various studies and arguments presented above, and the fact that *H. abyssinica* is an obligatorily outcrossing dioecious species with wind-induced seed and pollen dispersal, we developed the following three hypotheses for each of the objectives: (1) Genetic diversity is high within and low among populations; (2) genetic diversity is not correlated with population size and is not lost along recolonization routes; (3) no significant fine-scale spatial genetic structure is observed within populations.

Materials and methods

Sampling and DNA isolation

Twenty two naturally regenerated and three planted populations were surveyed from all regions where *H. abyssinica* is known to grow in Ethiopia (Fig. 1). A sampling spot was chosen at random from each of the naturally regenerated and planted populations. Twenty-three to twenty-four contiguous individuals per populations were sampled to allow the analyses of spatial genetic structure, particularly in the natural populations. The naturally regenerated populations represent twelve closed forest, eight woodland and two farmland ecosystems (Table 1). The planted populations were 2 – 15 years old and the seed sources for two of them (DKP and SMP, see Table 1) were known and among the 22 sampled natural populations. The sizes of the sampled naturally regenerated trees range from 3 m to 35 m in height and from 2.5 cm to 245 cm in diameter at breast height (DBH). The distances between trees within the same natural population range from 0.1 m to 730 m. The densities of the populations range from 0.7 to 75.7 and 1416 to 1912 individuals/ha for naturally regenerated and the planted populations, respectively (Table 1). Total population sizes were estimated for the natural populations by counting all trees with DBH above 2.5 cm.

Young leaves were collected, partially desiccated in paper bags before drying with silica gel and stored at room temperature until DNA isolation. The geographic coordinates of all the trees were recorded by using Garmin GPS®. Sexes of trees were identified only for those twelve populations with flowering trees at the time of the survey (Table 1). These data are used to compare genetic diversities between the two sexes. Genomic DNA was isolated from approximately 1cm^2 (ca. 20 mg) leaf materials following the DNeasy 96 kit protocol of Qiagen® (Hilden, Germany).

DNA restriction, PCR amplification and genotyping

The amplified fragment length polymorphism (AFLP) technique was employed to investigate patterns of genetic diversity, population differ-

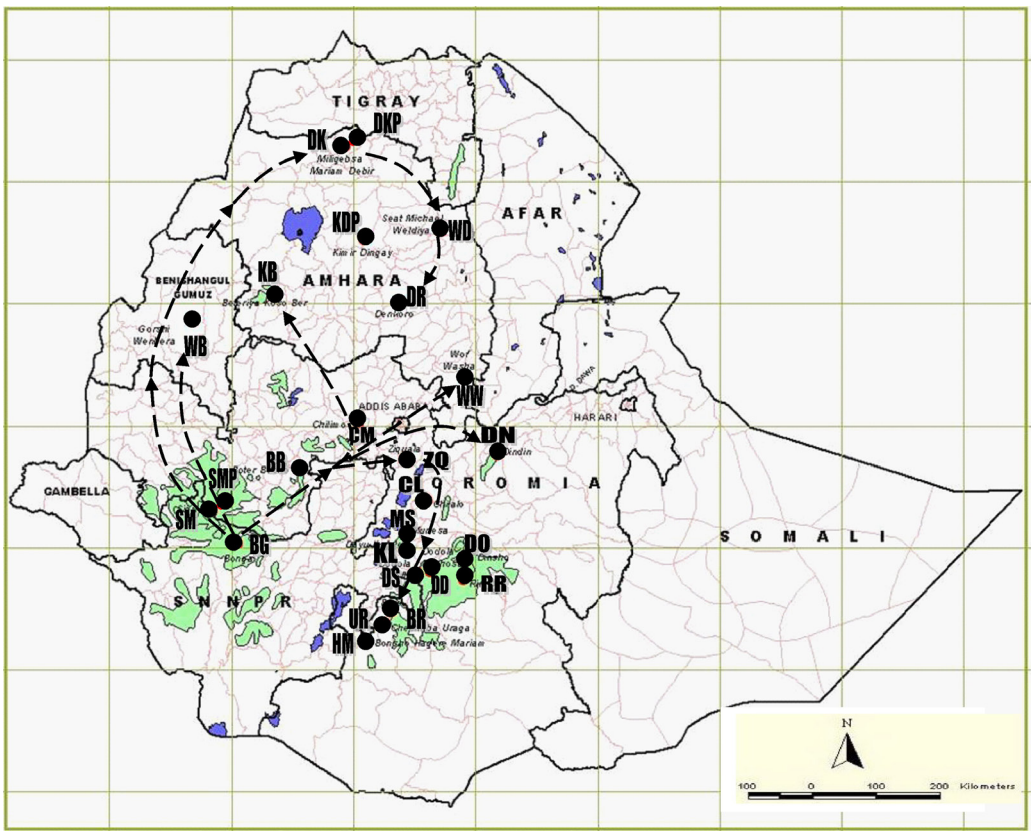

Figure 1 Distribution of *H. abyssinica* populations sampled from Ethiopia, represented by solid circles. Codes of populations follow Table 1. Broken lines with arrows indicate the putative recolonization route of *H. abyssinica* starting from the most likely source population BG as deduced from cpDNA analysis (Ayele et al. 2009). Shaded areas show extant closed forests. Source map: Assefa G (unpublished).

entiation and fine-scale spatial genetic structure in natural and some planted populations of *Hagenia abyssinica* (Bruce) J.F. Gmel from Ethiopia. The AFLP technique was chosen because of its cost-effective generation of data from a large number of loci distributed randomly across the genome and the ease to generate anonymous multilocus DNA profiles regardless of origin or complexity without prior sequence knowledge of the target species (Vos *et al.* 1995). AFLP has been used in various genetic studies; for example, genetic diversity (Cao et al. 2006), hybridization (Vasilyeva & Semerikov 2014), homoplasy (Vekemans et al. 2002), and molecular mapping studies (Ekramoddoullah & Liu 2008).

The laboratory protocol followed Vos *et al.* (1995) with some modifications. Genomic DNA was digested with two different restriction enzymes, a rare-cutter (*Eco*RI; 5'-G↓AATTC-3') and a frequent-cutter (*Mse*I; 5'-T↓TAA-3'), and short DNA fragments (adapters) were ligated to cohesive ends of the restriction fragments. Four µl genomic DNA (about 10 ng) were added to 6 µl restriction-ligation reaction containing 1 µl T4-Ligase buffer (10x), 1 µl NaCl (0.5 M), 0.5 µl BSA (1 mg/ml), 3 µl M± Adapter (5 pmol/µl), 0.6 E±

Table 1 Description of *H. abyssinica* populations sampled from the mountains of Ethiopia

Populations	Code	Latitude	Longitude	Altitude (m asl[a])	ARF[b] (ml[c])	Min T[d]	Max T[e]	H[f]	n[g]	N[h]	Density (ind/ha)	*Sex index
Debark-Mariam	DK	13°11’	37°57’	3013	1270	8.8	19.7	4	24	26	16	1
Debark-Plantation	DKP	13°12’	38°01’	3005	1270	8.8	19.7	4	24	-		NA
Kimir-Dingay plantation	KDP	11°48’	38°14’	-	1350	9.2	21.9	1, 6	24	-		NA
Woldiya Se’at Michael	WD	11°55’	39°24’	3112	908	NA	NA	6	24	120		NA
Kosso-ber	KB	10°59’	36°54’	2702	2381	12.9	27.4	1	24	60	52	NA
Denkoro	DR	10°52’	38°47’	3061	896	10.9	21.8	6	24	60	12.5	0.7
Wonbera	WB	10°34’	35°41’	2428	1622	NA	NA	5	24	60	75.7	NA
Wof-washa	WW	09°45’	39°44’	3159	941	6.1	19.9	1	24	45	5.4	NA
Chilimo	CM	09°05’	38°10’	2805	1114	11.5	25.8	1, 4	24	65	17.8	NA
Dindin	DN	08°36’	40°14’	2410	989	12.7	28,0	1	24	55	13.9	NA
Zequala Abo	ZQ	08°32’	38°50’	2856	1215	NA	NA	1	23	60	0.7	0.3
Boter-becho	BB	08°24’	37°15’	2772	1666	5.7	23.6	1	24	60	27.8	1.2
Chilalo	CL	07°56’	39°11’	2815	796	9.8	23,0	1	24	70	7.1	1.8
Sigmo plantation	SMP	07°55’	36°10’	2300	1837	11.4	21.6	4	24	-		NA
Sigmo	SM	07°46’	36°05’	2651	1837	11.4	21.6	4	23	60	23.8	NA
Munesa	MS	07°25’	38°53’	2459	1028	10.1	24.3	1	24	80	10	NA
Bonga	BG	07°17’	36°22’	2238	2217	11.9	26.6	4	24	80	5	0.9
Kofele	KL	07°11’	38°52’	2757	1305	7.7	20.1	1,2,3	24	110	12.5	1.8
Dinsho	DO	07°05’	39°47’	3117	1213	3.4	20.8	2	24	260	16.7	3
Doddola-Serofta	DS	06°52’	39°02’	2700	1074	6.7	24.3	2	23	75	10	2.9
Doddola-Dachosa	DD	06°52’	39°14’	3039	1074	6.7	24.3	2	24	5000	30.9	NA
Rira	RR	06°45’	39°43’	2725	736	NA	NA	2	23	170	10.5	NA
Bore	BR	06°17’	38°39’	2631	1526	8.3	18.8	1,2,3	24	100	9.1	1.1
Uraga	UR	06°08’	38°33’	2508	1228	8.3	18.8	2,3	24	70	13.9	0.7
Hagere Mariam	HM	05°51’	38°17’	2443	1228	12.3	23,0	2	24	55	4.8	0.9
Total									596	6741		

Note. Abbreviations: [a]masl - meters above sea level; [b]ARF - Mean Annual Rainfall; [c]ml - millilitres; [d]Min T - Mean minimum temperature; [e]Max T - Mean maximum temperature; [f]H - chloroplast haplotype (Ayele et al. 2009); gn - no. of samples analysed; [h]N - estimated population size; *sex index is determined from the relative numbers of male to female individuals for 26-50 individuals from each population; NA - not available. Source of climatic data: National Meteorological Agency Service (Ethiopia).

Adapter (5 pmol/µl) and to 2 µl restriction-ligation mix containing 0.2 µl T4-Ligase buffer (10x), 0.2 µl NaCl (0.5 M), 0.1 µl BSA (1 mg/ml), 0.08 µl *MseI* (10 U/µl), 0.6 µl *EcoRI* (10 U/µl) and 0.82 µl T4-Ligase (4 U/µl). The resultant solution was incubated at room temperature over-night (~16 hours). A pre-amplification PCR was run in a Peltier Thermal Cycler PTC-200 (MJ Research®), with a total volume of 15 µl containing 7.8 µl HPLC H_2O (high performance liquid chromatography water), 1.7 µl PCR buffer (10x), 1 µl dNTPs (2.5 mM), 0.25 µl of the pre-selective primer MO3 with selective nucleotide G (5 pmol/µl), 0,20 µl of EO1 with selective nucleotide A (5 pmol/µl), 0.06 µl Taq polymerase (Qiagen®) (5 U/µl) and 4 µl of the restriction-ligation reaction (diluted ~1:4). The pre-amplification PCR profile was 15 min. at 72 ˚C, followed by 20 cycles of 10 s denaturation at 94 ˚C, 30 s annealing at 56 ˚C and 2 min. extension at 72 ˚C, with a final extension step of 30 min. at 60 ˚C. A selective amplification was run with a total volume of 15 µl containing 8.11 µl HPLC H_2O, 1.6 µl PCR buffer (10x), 0.4 µl dNTPs (2.5 mM), 0.6 µl M67-Primer with selective nucleotides GCA (5 pmol/µl), 0.25 µl E41-Primer with selective nucleotides AGG (5 pmol/µl), 1.0 µl $MgCl_2$ (25 mM), 0.06 µl Taq polymerase (Qiagen®) (5 U/µl), and 3 µl pre-amplification product (diluted 1:10). The selective PCR profile was 15 min. initial denaturation at 94 ˚C, followed by 9 cycles of 30 sec. denaturation at 94 ˚C, 30 s annealing at 65 ˚C (reduced by 1 ˚C per cycle) and 2 min. extension at 72 ˚C, followed by 24 cycles of 30 s denaturation at 94 ˚C, 30 s annealing at 56 ˚C and 2 min. extension at 72 ˚C, with a final extension of 10 min at 72 ˚C. Aliquots of the selective amplification products were diluted (1:5) before electrophoretic separation. Two µl diluted selective PCR product was added to 12 µl HiDi formamide dye containing ~0.02 µl internal size standard (GS ROX 500, Applied Biosystems®), denaturated for two minutes at 90ºC, immediately cooled on ice, and separated on a capillary sequencer ABI 3100 Genetic Analyser (Applied Biosystems®).

Eighteen primer combinations (made up of four primers for EcoRI adapters and six primers for MseI adapters) were tested in different sets. The primer combination E41-M67 (5'-FAM-GAC TGC GTA CCA ATT CAG G-3', 5-GAT GAG TCC TGA GTA AGC A -3', respectively) showed a well-resolved banding pattern and a high degree of polymorphism. A total of 596 individuals (23-24 per population) were genotyped with this combination. Reproducibility tests were conducted on 15 samples randomly selected from each run. For these 15 samples, the whole AFLP procedure was done twice (restriction ligation, pre-selective and selective amplification). Only 100 percent reproducible loci were considered in the final analysis, resulting in 106 putative loci. Furthermore, three standard lanes, two containing the same individuals and one holding a negative control were run on each plate to compare the data from different runs and to check for the mobility of fragments.

Data analysis

Data were aligned with the internal size standard using GENESCAN 3.7 and fragments were scored with GENOTYPER 3.7 (Applied Biosystems®). Fragments with sizes ranging from 50-500 nucleotides (bp) were scored as present (1) or absent (0) and transformed to a 1/0 matrix. Each fragment was controlled and edited manually. Overall and gender-segregated genetic diversity (estimated as total diversity (H_t), within-population diversity (H_e) and among-population diversity (H_b)), percentage of polymorphic loci (PPL) at the 5 percent level, and coefficient of differentiation among-populations (F_{ST}) were computed using AFLP-SURV (Vekemans *et al.* 2002, available at http://www.ulb.ac.be/sciences/lagev/) following a Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky 1999). Gene flow (N_m) was estimated using the formula: $Nm = (1-F_{ST})/4F_{ST}$ (Slatkin & Barton 1989). Hardy–Weinberg equilibrium

was assumed in all computations. Partition of genetic diversity and the significance of the differences within and among-populations and different groups were estimated by an analysis of molecular variance (AMOVA) using ARLEQUIN Version 3.0 based on AFLP phenotypes (Excoffier *et al.* 2005; http://cmpg.unibe.ch/software/arlequin3). The different groups of the sampled populations that are used to examine the partitioning of genetic diversity are provided in Supporting Information 1. The fine-scale spatial autocorrelation analysis for 21 natural populations was performed with SPAGeDi 1.2 (Hardy & Vekemans 2002) using pairwise kinship coefficients (F_{ij}) between individuals plotted against pairwise distances (Hardy, 2003). The inbreeding coefficient is assumed to be 0 (as for dioecious species) following Hardy *et al.* (2006) and Tero *et al.* (2005). The significance of the spatial genetic structure (SGS) was tested by upper and lower bounds of the 95 percent confidence interval of F_{ij} defined after 10,000 random permutations of individuals among geographic locations. Eight distance classes were determined for all populations with one exception (DK, set to 4 classes) after series of tests in order to obtain a minimum of 30 pairs of individuals that lie within a given distance interval. The program NTSYS-pc 2.0 (Rohlf 1998) was used to calculate an UPGMA (Unweighted Pair Group Method with Arithmetic mean) tree to visualize genetic distances between populations. Additionally, a Mantel test was performed to examine the correlation between geographic and genetic distances. The Spearman's nonparametric correlation (Spearman 1907) was used to test for associations between genetic diversity and population size, tree density, distance from the putative original population (Bonga forest), and distance from the nearest population.

Results

Within population genetic diversity

The AFLP analysis of 596 samples from 25 populations of *H. abyssinica* resulted in a total of 106 unambiguously scorable putative markers in the range from 52 to 496 bps of which 97 (91.5%) were polymorphic. The percentage of polymorphic loci (PPL) within-populations ranged from 29.9 percent at Dodola Serofta (farmland/homestead population with a size of N = 75) and Uraga (located in a very small forest, N = 70) to 90.7 percent at Dinsho (located in a well-protected Park Forest, N = 260). Varying genetic diversities were observed at AFLP loci ranging from 0.139 at Dodola-Serofta to 0.362 at Dinsho with a mean genetic diversity of $H_e = 0.195$ (Table 2, 3). The largest remaining population DD (N ≈ 5000) showed only a moderate genetic diversity ($H_e = 0.173$, PPL = 36.1%). On the other hand, population DK with only 26 remaining individuals showed comparatively high levels of genetic diversity ($H_e = 0.217$, PPL = 45.4%). In general, closed forest populations harbored slightly more genetic diversity (mean $H_e = 0.207$) than woodland (mean $H_e = 0.190$) and farmland (mean $H_e = 0.172$) populations.

The planted populations harboured slightly different mean genetic diversities from their putative seed sources (H_e of DK = 0.217, H_e of DKP = 0.226; H_e of SM = 0.170, H_e of SMP = 0.146). The planted population DKP contained slightly higher genetic diversity than its putative parent population DK probably due to sampling bias and infusion of pollen to the putative parent population from external sources. On the other hand, the plantation SMP exhibited lower genetic diversity than its putative parent population SM most likely due to sampling bias.

There was no association between genetic diversity and actual population size (Spearman's nonparametric correlation r = -0.208, p = 0.353). Even though there are marked dif-

Table 2 Summary of within-populations genetic diversity and spatial genetic structure in *H. abyssinica* populations. The populations are sorted north to south.

Population code	Type of forest	Stand type	PPL[a]	H_e[b]	Density (ind/ha)	Max F(d)[c]	Max distance	Distance classes[d]	Distance (km) from nearest population
DK	woodland	Pure Hagenia	45.4	0.217	16	0.19	36	1	215.0 (WD)
DKP	plantation	plantation	47.4	0.226	na	na	na	na	na
KDP	plantation	plantation	39.2	0.183	na	na	na	na	na
WD	woodland	Hagenia-dominated mixed	43.3	0.194	na	na	na	na	na
KB	Closed forest	Hagenia-dominated mixed	45.4	0.206	52	0.06	15	1-2	141.2 (WB)
DR	Closed forest	Mixed, sparse Hagenia	39.2	0.189	12.5	ns	ns		131.7 (WD)
WB	Closed forest	Hagenia-dominated mixed	43.3	0.211	75.7	ns	ns		141.2 (KB)
WW	woodland	Hagenia-dominated mixed	41.2	0.189	5.4	ns	ns		141.7 (DN)
CM	Closed forest	Mixed, sparse Hagenia	37.1	0.192	17.8	0.09	31.6	1-3	98.8 (ZQ)
DN	Closed forest	Mixed, sparse Hagenia	48.5	0.212	13.9	ns	ns		136.3 (CL)
ZQ	Closed forest	Mixed, sparse Hagenia	45.4	0.205	0.7	ns	ns		78.3 (CL)
BB	Closed forest	Mixed, sparse Hagenia	44.3	0.213	27.8	0.07	18	1	122.2 (CM)
CL	woodland	Hagenia-dominated mixed	37.1	0.177	7.1	0.08	80	1-2	61.4 (MS)
SMP	plantation	plantation	33.0	0.146	na	na	na	na	na
SM	Closed forest	Mixed, sparse Hagenia	38.1	0.170	23.8	ns	ns		69.3 (BG)
MS	Closed forest	Mixed, sparse Hagenia	49.5	0.200	10	ns	ns		28.5 (KL)
BG	Closed forest	Hagenia-dominated mixed	46.4	0.198	5	ns	ns		69.3 (SM)
KL	Wooded grassland	Hagenia-dominated mixed	42.3	0.195	12.5	0.2	56	1	28.5 (MS)
DO	Closed forest	Hagenia-dominated mixed	90.7	0.362	16.7	0.21	44	1	37.5 (RR)
DS	Farm land	Pure Hagenia	29.9	0.139	10	0.12	64	1-2	22.3 (DD)
DD	Closed forest	Hagenia-dominated mixed	36.1	0.173	30.9	ns	ns		22.3 (DS)

Table 2 (continuation)

RR	Woodland	Hagenia-dominated mixed	36.1	0.169	10.5	0.06	52	1-2	37.5 (DO)
BR	Wooded grassland	Hagenia-dominated mixed	38.1	0.187	9.1	ns	ns		20.6 (UR)
UR	Closed forest	Mixed, sparse Hagenia	29.9	0.160	13.9	ns	ns		20.6 (BR)
HM	Farm land	Hagenia-dominated mixed	35.1	0.168	4.8	0.09	58	1	41.0 (UR)

Note. Abbreviations: [a]PPL - percent of polymorphic loci, [b]H_e - Nei's gene diversity, [c]F(d) - kinship coefficient averaged over distance classes, [d]distance classes for populations that showed family structures are indicated, na - not available, ns - not significant.

Table 3 Summary of the mean genetic diversity and population differentiation in subdivided populations of *H. abyssinica* for all populations and for the two chlorotype lineages

	H_t[a]			H_w[b]			H_b[c]			F_{ST}[d]		
	All	*LI*[e]	*LII*[f]	All	*LI*	*LII*	All	*LI*	*LII*	All	*LI*	*LII*
Mean	0.212	0.206	0.214	0.195	0.193	0.197	0.016	0.013	0.018	0.077	0.063	0.083
Upper 99%	limit									0.013	0.013	0.018
p										0.000	0.000	0.000

Note. Abbreviations: [a]H_t - total diversity, [b]H_w - within-population diversity, [c]H_b - among-population diversity, [d]F_{ST} - population differentiation, [e]*LI* - lineage I: DK, DKP, KDP, WD, DR, WB, SM, SMP and BG populations, [f]*LII* - lineage II: BB, BR, CL, CM, DD, DN, DO, DS, HM, KB, KL, MS, RR, UR, WW and ZQ populations; for population codes, see Table 1. Upper 99% limit - value of F_{ST} lying at the 1% rightmost part of the distribution under the null hypothesis, *p* - the probability of rejecting the null hypothesis.

ferences in genetic diversity for some populations, mean genetic diversities for the two sexes are similar (H_e = 0.207 ± 0.013 for male, H_e = 0.201 ± 0.019 for female). The two chloroplast lineages (see introduction) show only minor differences in mean (H_w) and total genetic diversity (H_t) at AFLPs (lineage I: H_w = 0.193 and H_t = 0.206, lineage II: H_w = 0.197 and and H_t = 0.214; Table 3).

Based on chloroplast DNA analyses, the putative entry point of *H. abyssinica* into Ethiopia during recolonization is located in the southwestern mountains of Ethiopia (Ayele et al. 2009). The recolonization routes of the species were reconstructed from southwest to the north, to the east and to the south (Ayele et al. 2009, see also Fig. 1). There was no association between distance from the putative original population and genetic diversity (H_e, PPL) of populations (Spearman's nonparametric correlation r = -0.205, p = 0.186) and thus no indication of loss of genetic diversity during recolonization.

Partitioning of genetic diversity among populations

High mean within-population variation (H_e = 0.195) and moderate population differentiation (F_{ST} = 0.077, $p < 0.001$) was observed (Table 3). The differentiation between populations within the two chloroplast lineages (Ayele et al. 2009) was similar (lineage I: F_{ST} = 0.063 p< 0.001, lineage II: F_{ST} =0.083, $p < 0.001$) (Table 3). The estimated number of migrants per generation (N_m) as a parameter characterizing gene flow among populations computed for all populations based on F_{ST} was 3.

The analyses of molecular variance (AMOVA) performed for all populations revealed

Table 4 Partitioning of AFLP variation among *H. abyssinica* individuals in Ethiopia computed by analysis of molecular variance (AMOVA)

Source of variation	*df*[a]	*SS*[b]	*Vc*[c]	*Pv*[d]	*Ls*[e]
Among-populations	24	557.42	0.71554	10.40	***
Within-populations	571	3521.63	6.16748	89.60	***
Among ecosystem groups	4	89.92	-0.00874	-0.13	ns[f]
Among-populations	20	467.50	0.72178	10.49	***
Within-populations	571	3521.630	6.16748	89.64	***
Among geographic groups	3	105.427	0.09441	1.40	ns
Among-populations	21	451.993	0.64411	9.30	***
Within-populations	571	3521.63	6.16748	89.30	***
Among stand groups	3	64.062	-0.01630	-0.24	ns
Among-populations	21	493.358	0.72650	10.56	***
Within-populations	571	3521.63	6.16748	89.67	***
Among tree seed zones groups	12	311.133	0.18654	2.70	ns
Among-populations	9	168.756	0.52310	7.50	***
Within-populations	502	3132.63	6.24030	89.8	***
Among chloroplast lineage groups	1	37.525	0.05421	0.78	*
Among-populations	23	519.895	0.68950	9.98	***
Within-populations	571	3521.630	6.16748	89.24	***
Among sex groups	1	3.702	-0.08835	-1.34	ns
Among-populations	22	270.164	0.70368	10.64	***
Within-populations	193	1157.245	5.99609	90.69	***

Note. Abbreviations: [a]*df* - degree of freedom, [b]*SS* - sum of squares, [c]*vc* - variance components, [d]*pv* - percent variation, [e]*ls* - level of significance.

that 10.4 percent of the total variation was attributed to the differences among populations. Very low proportions of the total variation were distributed among groups representing different ecosystems, geographic regions, forest stands, tree seed zones and the two sexes (Table 4). Only differentiation among chloroplast lineages was significant but very low (PV = 0.78%, $p < 0.05$). When partitioned by different groups, the level of genetic differentiation among-populations is similar ranging from 7.5 percent to 10.6 percent (Table 4). No private fragment or fragment fixed in only one population was detected.

Relationships among populations

The pairwise Nei's genetic distance matrix (Supporting Information 3) among 25 populations exhibits genetic differences of less than 7 percent for each pairwise comparison. The UPGMA dendrogram calculated from Nei's genetic distances (Nei 1978) does not reflect the geographic origin of the populations (Fig. 2). Also, the planted populations DKP and SMP were not clustered with their putative parent populations DK and SM, respectively (Fig. 2). Effect of random variation due to sampling and possible infusion of pollen to the putative parent populations from external sources might explain this observation. A test of association between geographic and genetic distances (Mantel test) showed a very low and non-significant correlation ($r = 0.14607$, $p = 0.9024$). For example, the highest genetic distance was observed between populations RR and DO (0.0669) that are geographically close (37.5 km air distance) but separated by a big

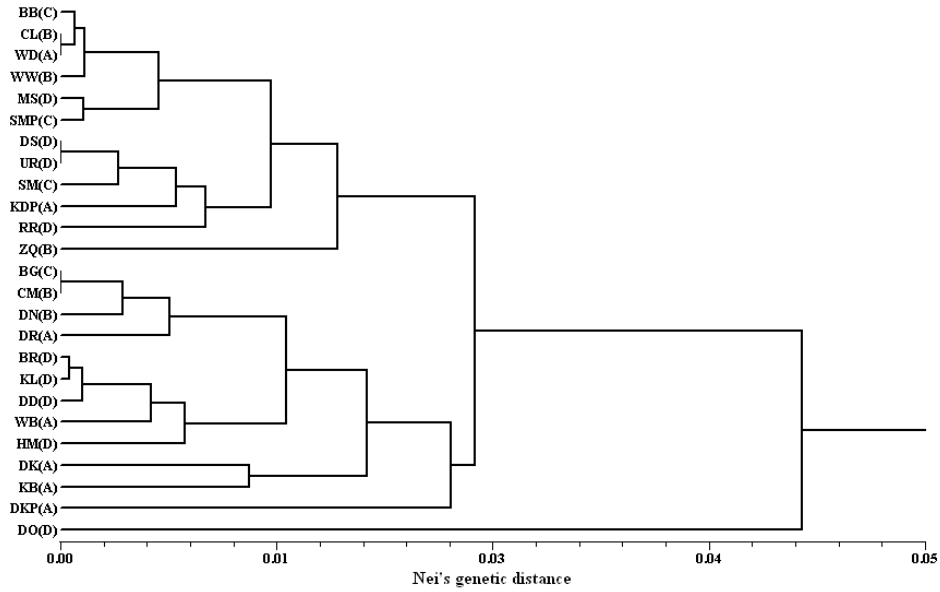

Figure 2 UPGMA tree drawn from Nei's (1978) genetic distances computed from AFLPs. Population codes follow Table 1. Letters in parenthesis designate geographic regions: A - northern region, B - central region, C - southwestern region, D - southern region.

mountain embracing the second highest peak in the country, Tulu Dimtu. On the other hand, the three pairs of populations with the lowest genetic distances (from 0 to 0.0005) between them (WD and WW, WD and BB and KL and BR) are widely separated (240 km, 452 km and 103 km, respectively), with some small mountains between them.

Fine-scale spatial genetic structure

In general, most populations from farmlands, wooded grasslands and woodlands (6 out of 8) showed significant spatial genetic structure up to greater distances (36-80 m) whereas only 4 out of 13 closed forest populations showed family structures at smaller distance classes (15-44 m) (Table 2; Supporting Information 4). No SGS was observed in the largest remaining *H. abyssinica* population (DD) in Ethiopia (~ 5000 individuals) while significant SGS was observed in the second largest population DO (N = 260), harboring the highest genetic diversity. In most (7 out of 10) of the small populations (N = 55-80) of the closed forest type, no SGS was observed (Table 2). Density, population size and distance from the nearest population were not associated with the kinship coefficient averaged over distance classes F(d) of 21 natural populations (for example, Spearman's nonparametric correlation coefficient (r) for density = -0.065, p = 0.391).

Discussion

Genetic diversity and population differentiation

Effective gene flow through pollen and possibly seed is likely the main factor contributing to low population differentiation and varying genetic diversity within populations of *H. abyssinica*. Results confirm the first hypothesis that predicted high variation within populations and low differentiation among popula-

tions. Feyissa et al. (2007) reported higher mean genetic diversity (0.30) in 12 populations of *H. abyssinica* from central and southern regions of Ethiopia at 84 polymorphic ISSR markers. They also found a higher coefficient of differentiation (G_{ST} = 0.25) than the present work. Different marker systems, fewer populations and small sample sizes in their study might have contributed to the differences from our results.

No decreasing genetic diversity was observed along recolonization routes, due to effective gene flow that counteracts effects of genetic drift (hypothesis 2). A general trend of increasing genetic diversity away from refugia was observed in European beech based on isozymes (Comps et al. 2001), suggesting a gain in genetic diversity during recolonization due to gene flow, population admixture effects and selection. Petit et al. (2003) also reported that the mixing of colonization routes and increased levels of seed flow resulted in increased intrapopulation diversity away from refugia in some European woody species at maternally inherited markers. In contrast, *Lobelia giberroa*, which entered Ethiopia also from the south (Kebede et al. 2007), *Carpinus betulus* (Betulaceae) in Europe (Coart *et al.* 2005) and *Ptercarpus officinalis* (Fabaceae) in the Caribbean (Rivera-Ocasio et al. 2002), exhibited decreasing diversity during recolonization at AFLP markers. As *H. abyssinica* is presumably a predominantly wind-pollinated canopy tree, pollen most likely disperse over long distances contributing to the maintenance of comparatively high levels of genetic diversity. This might explain the lack of association between genetic and geographic distances at AFLPs.

The observation of higher diversity in closed forests, the location of the population with highest diversity in a well-protected forest, and the observation of the lowest diversity in two farmland populations suggest human impact on populations reduced genetic diversity. Genetic diversity was not correlated with sizes of extant populations (hypothesis 2). This result might be due to recent reductions of population sizes due to human impact, which did not yet diminish genetic diversity, and due to efficient gene flow among populations in the past. Phylogeographic analyses of the 25 populations at cpDNA revealed two chloroplast lineages (Ayele et al. 2009). Most likely, lineage I originated from lineage II by a deletion in a specific chloroplast region. We would expect a lower genetic diversity in populations with the derived chloroplast haplotypes of lineage I. However, lineage II exhibited only slightly higher diversity than lineage I. Also, there was no significant differentiation between chloroplast lineages at AFLPs presumably due to efficient long-distance gene flow through pollen.

Comparison of genetic diversity with other species

In her review of intraspecific genetic diversity in plant species at nuclear DNA markers, Nybom (2004) reported a slightly higher mean within-population genetic diversity (H_{pop}) of 0.22 at RAPDs, 0.23 at AFLPs and 0.22 at ISSRs than the mean genetic diversity of *H. abyssinica* (H_e = 0.195) at AFLPs. Studies based on AFLP markers are limited to a few tropical tree species and information on the method of estimating H_e is missing in most of the cases, making comparisons difficult. The mean genetic diversity of *H. abyssinica* is moderate compared to other species for which the same method for the estimation of allele frequencies was applied (Supporting Information 5). Wider natural distribution of *Cordia africana* (H_e= 0.287) and *Juniperus procera* (H_e= 0.269) in Ethiopia and the effective dispersal of seeds of *C. africana* by animals most likely explain the higher diversity observed in these two species as compared to that of *H. abyssinica* (H_e= 0.195) (Supporting Information 5).

Most studies investigating genetic differentiation among populations of wind-pollinated forest trees were conducted in temperate and boreal forests. Most species investigated were of the Pinaceae and Fagaceae families, and un-

like *H. abyssinica*, populations were typically large, more or less continuous, and characterized by high densities. Most of these genetic inventories in stand-forming, common Pinaceae (e.g. *Pinus* spp., *Picea* spp., *Pseudotsuga menziesii*; Lagercrantz & Ryman 1990, Li & Adams 1989) and Fagaceae (*Fagus, Quercus*; e.g. Comps et al. 2001) revealed very low differentiation (Austerlitz et al. 2000). Differentiation was higher for insect-pollinated species with a scattered distribution both in temperate (e.g. *Sorbus torminalis*; Demesure et al. 2000) and tropical (e.g. *Shorea* spp.; Cao et al. 2006) forests (Hamrick et al. 1992). Since it is predominantly wind-pollinated, has a very scattered distribution and small populations at least in Ethiopia, *H. abyssinica* is a species with a rare combination of life history traits. The differentiation among *H. abyssinica* populations is higher in comparison to most common wind-pollinated species occurring in high density, but lower than for most insect pollinated species occurring in low density.

Fine-scale spatial genetic structure

Despite the dispersal of seed and pollen predominantly by wind, significant spatial genetic structure was observed within nearly half of the populations of *H. abyssinica*, reflecting restricted gene flow within populations. Positive values of F_{ij} above the 95% confidence interval were found at short distances, indicating higher genetic relatedness among neighboring individuals than among random pairs of individuals, whereas negative values of F_{ij} occurred at larger distances, showing isolation-by-distance within a population (Tero et al. 2005). Significant spatial genetic structure in *H. abyssinica* extends up to 80 m. This result allows us to reject the hypothesis that predicts absence of fine-scale genetic spatial patterning in *H. abyssinica*. Even though seeds are very light, restricted dispersal of diaspores is likely to be the principal cause of spatial structures, assuming that seed dispersal in *H. abyssinica* is more restricted than pollen dispersal as in most other trees. While there was no association between tree density, population size or distance from the nearest population and the occurrence of wide-ranging SGS, significant fine-scale SGS was observed more frequently in farmlands and open woodlands as compared to closed forests.

Conclusion

Despite the relatively recent recolonization of Ethiopia by *H. abyssinica* that has been suggested by fossil pollen data and the small population sizes, the AFLP analysis detected surprisingly high genetic diversity within populations with considerable differences in H_e between populations, and low but significant genetic differentiation among populations. Since even a restricted number of migrants per generation is sufficient to counteract genetic differentiation (Wright 1931), the effect of recent recolonization is not reflected in the levels of genetic diversity. The observed variation does not reflect clinal variation patterns. Consequently, (1) only a seed zone approach is questionable to conserve genetic diversity; (2) it is difficult to capture optimal variation for conservation and tree improvement based on approaches to sample ecological and/or geographic zones without consideration of genetic variation; (3) due to significant genetic differentiation observed among populations, it is necessary to collect seeds from as many populations as possible for gene bank storage, establishment of provenance trials, establishment of *ex situ* conservation stands, and for forestation programs. The very high genetic diversity in some populations calls for the need to conserve the observed variability. The moderate to high intraspecific variation and a wide vertical distribution of the populations (2200 to 3200m asl) may suggest that *H. abyssinica* might have occupied wider areas in the past. The extant populations, on the other hand, harbor quite high levels of genetic diversity despite of their small sizes. Nonetheless, our

data suggest that human impact in the form of selective removal of trees conversely affects genetic diversity, as observed in the two farmland populations. In general, the investigation of genetic diversity at AFLPs covering the natural distribution range in Ethiopia enhanced our understanding of the phylogeography, genetic structure and the forces shaping genetic variation patterns in *H. abyssinica*.

Further work on the intraspecific genetic variation and palynological investigations in other African countries where *H. abyssinica* is known to grow is recommended to fully understand the recolonization history and to identify the refugia of the species. Fragmentation might have caused isolation of populations that were previously interconnected through gene flow, and it is therefore recommended to study extant gene flow in the few remaining relic populations.

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Supporting Information

The online version of the article includes Supporting Information:

Supp. Info. 1. The grouping of the sampled *H. abyssinica* populations that were used to examine the partitioning of genetic diversity at AFLP loci.

Supp. Info. 2. A female *Hagenia* tree demonstrating a very good quality timber from Uraga population, South Ethiopia.

Supp. Info. 3. Pairwise matrix showing Nei's genetic distance among 25 populations of *H. abyssinica* from Ethiopia.

Supp. Info. 4. Correlograms showing kinship coefficient (F(d)) averaged over distance classes and plotted against the maximum distances of 8 distance classes from AFLPs of 21 natural populations of *Hagenia abyssinica*.

Supp. Info. 5. Comparison of the genetic diversity of *H. abyssinica* with other species at AFLPs.