Hydrological connectivity and vegetative dispersal shape clonal and genetic structure of the emergent macrophyte Cyperus papyrus in a tropical highland lake (Lake Tana, Ethiopia)

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Hydrological connectivity and vegetative dispersal shape clonal and genetic structure of the emergent macrophyte *Cyperus papyrus* in a tropical highland lake (Lake Tana, Ethiopia)

Addisie Geremew · **Ludwig Triest**

Abstract Understanding populations' genetic connectivity is critical for conservation and management measures. Most population connectivity studies conducted in aquatic plants have been centered on linearly arranged systems wherein a cline in genetic diversity is expected to the downstream. However, non-linear systems and lakes characterized by hydrological mixing, circulation, and strong discharge might not support this assumption. Considering these processes and the mixed reproductive mechanisms of *Cyperus papyrus* (L.), we assessed the relative role of hydrological connectivity and vegetative dispersal in shaping the clonal and genetic structure of the species. Four hundred and two papyrus individuals from 13 populations fringing Lake Tana, Ethiopia, were genotyped using 15 microsatellite markers. Despite the genetic bottleneck detected, papyrus populations maintained moderate levels of clonal ($R = 0.48$) and genetic diversity ($H_E = 0.40$). We found a significant within-lake level of genetic differentiation ($F'_{ST} = 0.16$; $D_{EST} = 0.10$). Bayesian analysis assigned individuals to three non-panmictic genetic clusters. Evidences of long-distance vegetative dispersal and asymmetrical contemporary migration were detected among *C. papyrus* populations. The patterns of the genetic structure, clonal dispersal, and migration rate partly correspond to the surface flow. Overall, hydrological connectivity and vegetative dispersal shape the clonal and genetic structure as well as the connectivity of *C. papyrus* populations in Lake Tana.

Keywords Microsatellite · Lake Tana · Clonal dispersal · Hydrological connectivity · *Cyperus papyrus*

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Introduction

Papyrus swamps dominated by *Cyperus papyrus* L. are biologically productive and dynamic land–water ecotones (Gaudet, 2014). They provide critical ecosystem services, including sequestering carbon (Mitsch et al., 2010), stabilizing sediment load (Jones & Humphries, 2002; Wondie, 2010), supporting biodiversity (Mnaya

& Wolanski, 2002; Maclean et al., 2011), and supplying raw materials for handcrafting (Terer et al., 2012). However, human activities, for example, agricultural expansion, urbanization, and over-exploitation (Owino & Ryan, 2007; Wondie, 2010), and global climate change would result in water level fluctuations threatening papyrus wetlands (Gaudet, 2014).

Cyperus papyrus is a fast-growing, wind-pollinated, rhizomatous, perennial C4 emergent freshwater sedge native to Africa (17°N and 29°S, up to 2335 m) (Terer et al., 2012). It has also been introduced and naturalized in few other parts of the world (Perbangkhem & Polprasert, 2010; Terer et al., 2012). Reproduction in this species occurs both vegetatively by means of rhizome extension and generatively through production of light and buoyant seeds that are primarily and secondarily dispersed by wind and water, respectively (Terer et al., 2015). A seismic survey that coalesced with a paleo-ecological analysis showed that *C. papyrus* has occupied Lake Tana (Ethiopia), the tropical highland lake between 16,700 and 15,500 years (Lamb et al., 2007). Since then, human impacts as well as landscape changes in the lake and fringed swamps have taken place (Teshale et al., 2001; Tarekegn & Tadege, 2007). For instance, due to urbanization, agricultural expansion and dam construction between 1986 and 2013 over 52% of wetlands surrounding this lake have been changed into other land use forms (Goshu & Ayanlem, 2017; Hassen & Assen, 2017). Consequently, as has been the case elsewhere in Africa (Owino & Ryan, 2007; Maclean et al., 2011), only small, patchy, and fragmented pockets of *C. papyrus* populations remain in the Lake Tana basin (Teshale et al., 2001; Dejen et al., 2004). Consequently, papyrus populations might have passed through several bottlenecks that accelerate the stochastic loss of genetic diversity. In turn, this would affect their resilience to perturbation, decrease their fitness by favoring inbreeding (Frankham et al., 2002; Reynolds et al., 2012), and eventually influence ecosystem function and services (Hersch-Green et al., 2011). Alternatively, only the most resilient genets remain after passing through a bottleneck, potentially with some degree of inbreeding, although the species might never have harbored a vast amount of genetic diversity (Arnaud-Haond et al., 2010). Bearing in mind the possible long-term and short-term consequences that could arise from a genetic bottleneck,

exploring the genetic and clonal diversity is imperative for conservation measures.

Maintaining populations genetic connectivity is a critical issue in terms of both conservation and restoration. However, human-driven wetland modifications hamper populations' genetic connectivity by impeding dispersal pathways (James et al., 2013). Ultimately, this reshuffles the genetic diversity and structure (Virtanen & Oksanen, 2007; Kendrick et al., 2016) as well as population resilience (Ishiyama et al., 2015). In aquatic plants with mixed reproduction modes, genetic connectivity is sustained by the dispersal of either vegetative or generative propagules. The former is ecologically valuable when generative dispersal is ineffective under environmental constraints (Tatarenkov et al., 2005). A variety of dispersal vectors such as wind, water, and animals (Soons et al., 2008), mediate the dispersion of the propagules of aquatic plants. In most cases, a combination of these dispersal agents operates together (Ozinga et al., 2004). Water-mediated dispersal (hydrochory) has the potential to change the genetic structure and diversity of aquatic plants (Nilsson et al., 2010) by promoting long-distance propagule transport. Local seed rain and dispersal have been shown to sustain the genetic and clonal diversity of *C. papyrus* in Lake Naivasha, Kenya (Triest et al., 2014). However, papyrus swamps in a hydrologically dynamic lake can detach and form floating islands that are further dispersed by wind and wave action (van Duzer, 2002; Morrison & Harper, 2009). Yet, the extent to which water-mediated clonal dispersal or drifting papyrus mats shape the genotypic diversity and connectivity of *C. papyrus* within a lake or catchment system remains unknown, although it has been demonstrated that within-lake genetic differentiation (e.g., Lake Victoria and Lake Naivasha) is lower than between-lake differentiation (Terer et al., 2015). Within-lake dispersal processes, therefore, can be reflected in the clonal and genetic structure of papyrus fringes.

Several gene flow studies on aquatic plants in a linear system expected to have a cline in genetic diversity to populations in the downstream by assuming a stepping-stone gene flow model (Pollux et al., 2009; Mitsui et al., 2010; Triest et al., 2010; Triest & Fenart, 2014). However, this is not the case when dispersal mechanisms other than hydrochory are involved or for populations in a non-linear

configuration (Tero et al., 2003; Hu et al., 2010; Honnay et al., 2010; Wei et al., 2015). Moreover, hydrodynamics is an important factor promoting gene flow among populations in marine ecosystems (White et al., 2010; Ruiz-Montoya et al., 2012). Nevertheless, its impact on emergent macrophytes under large freshwater lakes has been investigated to a much lesser extent.

The hydrodynamics of Lake Tana (Ethiopia) have been extensively studied (Kebede et al., 2006; Chebud & Melesse, 2009; Dargahi & Setegn, 2011; Dessie et al., 2014). The overall hydrodynamics of this lake resemble a closed system of a shallow reservoir with an overflow type of outlet (Dargahi & Setegn, 2011) and short periods of thermal stratification due to partial hydrological mixing (Dejen et al., 2004). Dargahi and Setegne (2011) indicated that energy induced by both rivers inflow and outflow, wind, and convective mixing has controlled the hydrological mixing processes. In addition, while the flow structures are composed of major surface circulation in a clockwise direction and a jet flow towards the lake outlet, a minor secondary transient flow occurs near the various islands and the shoreline vegetation (Dargahi & Setegn, 2011). These authors further noted that the prominent winds from the northeast and east of Lake Tana drive the flow south and southwest direction, respectively. However, the flow at the outlet region splits into two distinct zones owing to Debre Mariam Island, one to the east side leading into the outlet and a second to the west side. In view of the potential hydrochorous dispersal of *C. papyrus* seeds (Terer et al., 2015) and floating papyrus mats (van Duzer, 2002) as well as the underlying hydrodynamic and single outflow (Blue Nile River) regulated by a weir, the lake could be an ideal system to examine the role of hydrological connectivity in shaping papyrus populations genetic diversity, structure, and connectivity. For example, studies have shown that ocean currents and circulation patterns sustain if not drive the genetic connectivity and structure of coastal macrophytes (Ruiz-Montoya et al., 2015; McMahon et al., 2015) and mangroves (Pil et al., 2011; Wee et al., 2014) by influencing long-distance propagule dispersal, and the magnitude and direction of gene flow (van Dijk et al., 2009; Ruiz-Montoya et al., 2012). However, two scenarios of gene flow should be tested in a lake where hydrological mixing (Dejen et al., 2004) and surface circulation counteract the strong outflow (Dargahi &

Setegn, 2011). The first scenario would be that hydrological mixing and within-lake circulation govern the gene flow such that a uniform gene pool could be expected. Alternatively, provided that the effect of the lake surface outflow is stronger than the overall circulation, the genetic diversity would accumulate near the outlet, thereby enhancing genetic structuring.

In the present study, we applied fifteen polymorphic microsatellite markers to explore the role of hydrological connectivity and vegetative dispersal in shaping clonal and genetic diversity, structure, and connectivity of thirteen *C. papyrus* populations in and bordering a large tropical highland (Lake Tana, Ethiopia). We explicitly addressed the following research questions: (i) Is there unambiguous evidence of within-lake clonal dispersal and, if so, over which distances? (ii) Do the genetic structure and migration patterns follow the surface flow pattern? (iii) Is there higher genetic diversity in a population at the outlet (Blue Nile River) under regulated flow? (iv) Are there signs of recent population genetic bottlenecks?

Materials and methods

Sampling sites and sampling approach

This study was conducted in papyrus swamps in and bordering Lake Tana, the largest freshwater body (an area of 3,200 km^2) situated on the basaltic plateau of the northwestern Ethiopian highlands (Fig. 1). Lake Tana, which was formed by the volcanic jamming of rivers down the Quaternary basalt flow (Vijverberg et al., 2009), is composed of hydrological networks of rivers, of which the Gilgel Abay, Reb, Gumera, and Megetch are the perennial rivers that contribute more than 93% of the inflow (Kebede et al., 2006). The lake and the adjoining floodplains are designated as biosphere reserves and global lake regions (LakeNet, 2004) for wetland and biological diversity conservation. Its catchment comprises dry evergreen Afromontane forest, grassland complexes, swamps, floodplains, and shoreline vegetation fringed by emergent macrophytes (Friis et al., 2010). The shoreline and swamps are dominated by monotypic stands of the paper reed (*C. papyrus*) and the common cattail (*Typha latifolia*). The lake could have had a prominent place in subsistence and cultural history of ancient inhabitants (Lamb et al., 2007) and it was the trade
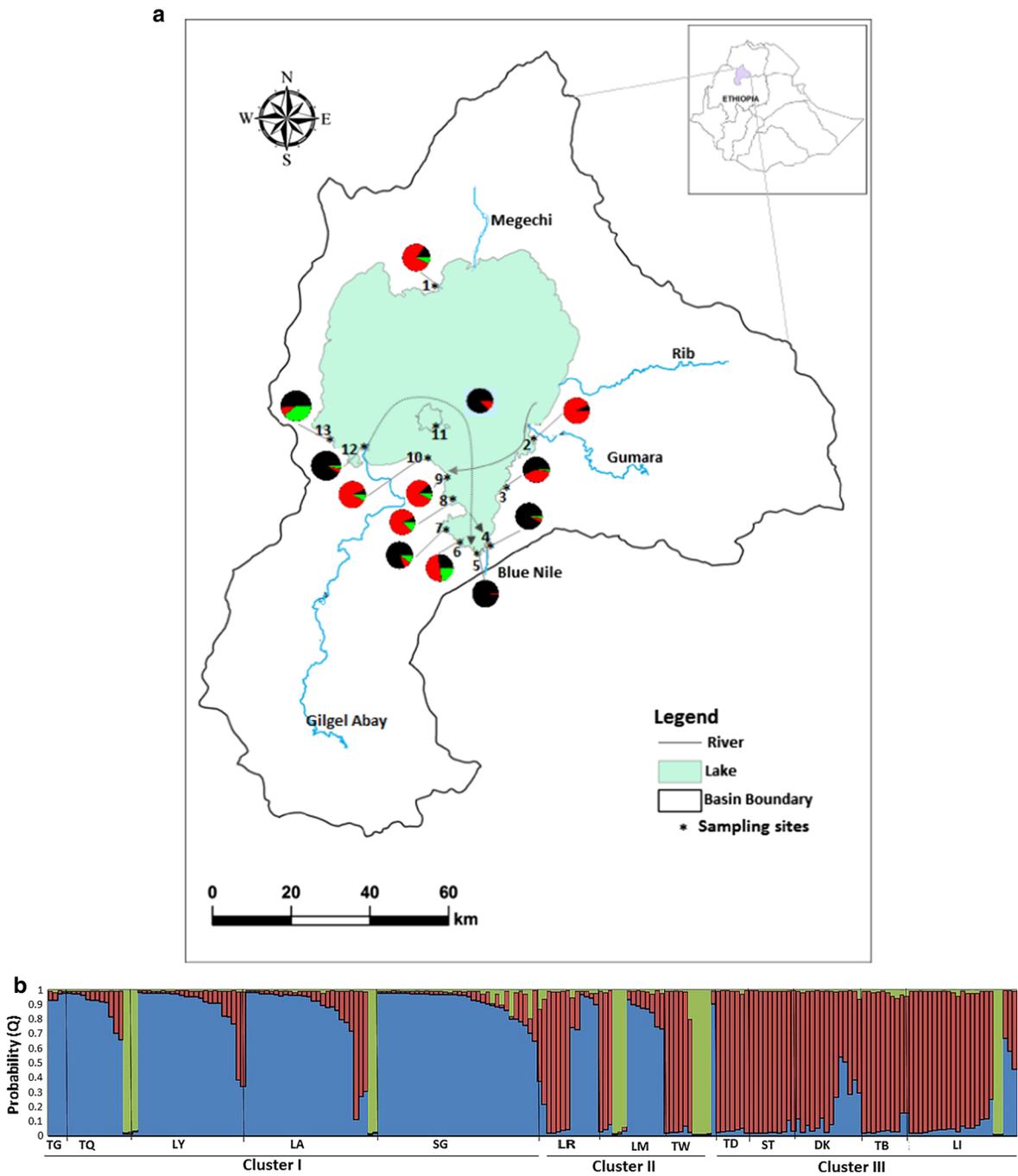
Fig. 1 a Locations of sampling sites in the Lake Tana (population's number and abbreviations are given in Table 1). Major surface flow is shown schematically (Dargahi & Setegn, 2011). Dotted line represents surface flow along the flow of Blue Nile mainly from the northern and southwestern Tana and dashed line represents surface currents from the Eastern Tana. The pie charts indicate the probability of the three genetic groups' ($K = 3$) distribution in each sample sites based on the STRUCTURE analysis. b The bar plot visualizes the individual's estimated membership fraction in each of the population clusters

Table 1 Clonal and genetic diversity of 13 *C. papyrus* populations over 15 nuclear microsatellite loci

Sampling area and abbreviations	Sample no.	Genotypic diversity							Genetic diversity						Bottleneck
		N	G	R	$C(\beta)$	D	ED	AC	A	A_r	PA	H_O	H_E	F_{IS}	P-sign
Gorgora (TG)	1	30	3	0.07	–	0.13	–	0.021[ns]	–	–	–	–	–	–	–
Tana Qirqos (TQ)	2	30	13	0.41	1.45	0.85	0.64	0.094[ns]	1.93	1.93	1	0.52	0.32	− 0.59	0.0002*
Robit (LR)	3	30	13	0.41	1.41	0.84	0.68	0.484**	2.53	2.53	0	0.39	0.36	− 0.04	0.0549
Debre Mariam (TD)	4	30	6	0.17	1.13	0.53	0.40	0.433*	*2.33*	*2.17*	*3*	*0.34*	*0.30*	*− 0.07*	*0.0250**
Southern tip (ST)	5	30	8	0.24	1.42	0.83	0.80	0.598**							
Michael (LM)	6	30	14	0.45	1.31	0.81	0.45	0.094[ns]	3.60	3.52	2	0.35	0.41	0.18*	0.6871
Infranz (LI)	7	30	22	0.72	2.13	0.98	0.77	0.215**	3.47	3.20	2	0.38	0.41	0.10	0.5548
Yganda (LY)	8	30	27	0.90	3.32	0.99	0.69	0.094*	3.60	3.15	7	0.35	0.42	0.18*	0.1514
Ambo-Bahir (LA)	9	30	27	0.90	4.91	0.99	0.00	0.065*	3.07	2.67	0	0.30	0.37	0.19*	0.3193
Sekelet Georgis (SG)	10	45	35	0.77	2.23	0.99	0.84	0.166**	3.60	2.81	13	0.37	0.38	0.01	0.4452
Deke Island (Dk)	11	27	15	0.55	1.64	0.92	0.74	0.276**	2.20	2.30	0	0.36	0.30	− 0.14	0.0415*
Upper Blue Nile (TB)	12	30	11	0.35	1.70	0.90	0.88	0.591**	*3.47*	*3.22*	*3*	*0.33*	*0.44*	*0.27**	*0.0320**
Womberya (TW)	13	30	8	0.24	1.42	0.85	0.75	0.011[ns]							
Mean/Total		402	202	0.48	–	0.80	0.64	–	2.98	2.79	31	0.36	0.37	0.01	–
SE									0.11			0.02	0.01		

N, number of samples; *G*, number of distinct MLGs; *R*, clonal richness; *C(β)*, Pareto coefficient; *D*, Simpson diversity index; ED, Simpson Evenness; AC, aggregation coefficient; *A*, number of alleles; *A*$_r$, allelic richness; PA, number of private alleles; *H*$_O$, observed heterozygosity; *H*$_E$, expected heterozygosity; *F*$_{IS}$, inbreeding coefficient and *P*-sign, significance value of sign test for two-phased and stepwise mutation model bottleneck test

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and ns = non-significant (–): parameter not calculated because of few genets. The italic values represented the pooled populations to compute genetic diversity metrics

route between the Aksum and the Blue Nile further south back to the sixth century (Henze, 2000). The increasing population coupled with the amplified demand for settlement and farming create a pressure on the shoreline vegetation and alter the hydrodynamic conditions of the lake (see Wondie, 2010; Dargahi & Setegn, 2011; Minale & Rao, 2012; Dessie et al., 2014).

We selected thirteen papyrus swamps (Fig. 1a, Table 1) by primarily considering the hydrological connectivity that influences species migration and dispersal (Morris et al., 2011), the area covered, and additionally the human impact (agricultural encroachment and over-exploitation). The swamps are hydrologically connected throughout the year, except for a minor drawdown from the land toward the lake during the dry season (e.g., LR, LY, and LM, Table 1 and Supplementary Table 1). In some instances, despite harvesting pressures (e.g., LA, SG, and LI), the papyrus populations are found to have a contiguous distribution. In contrast, the remaining sites are patchy and disturbed. During the summer of 2014, a total of 402 *C. papyrus* individuals were sampled at thirteen swamps in and around Lake Tana. At every sampling site, shoots (umbels) of 30–45 individuals were randomly collected at intervals of 3 m along a 90–120 m transect depending on the density and area covered. The plant samples were dried at room

temperature and then stored in bags filled with silica gel prior to the DNA extraction.

DNA extraction and microsatellite genotyping

Genomic DNA was extracted using an E.Z.N.A.® *SP* plant DNA Miniprep Kit (OMEGA BIO-TEK, Inc.) from 20 mg of ground dried leaves. We used fifteen fluorescently labeled microsatellite primers developed in two multiplex sets by Triest et al. (2013) and Geremew et al. (2015) to amplify the sample DNA (Supplementary Table 2). The polymerase chain reaction (PCR) profiles are as described by Triest et al. (2013). The amplified products were separated using an ABI 3730xl genetic analyzer, while the fragment sizes were determined according to the internal size standard of GeneScan 500 LIZ (Macrogen Seoul, Republic of Korea). The allele sizes of each microsatellite locus per individual were identified and scored using GeneMarker® v2.4.1 (SoftGenetics, State College, PA, USA). Prior to the analysis, the genotyping error and presence of null alleles, stuttering, and large allele dropout were tested using Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004) with 10,000 randomizations (95 and 99% confidence levels).

Data analysis

Clonal diversity and arrangement

Genotype data of 402 individuals from thirteen *C. papyrus* populations were used for the clonal analysis. Since *C. papyrus* is a clonal plant, the number of shoots sampled in a site corresponds to distinct genotypes or else they may represent duplicate genotypes collected from the same clone. To account and correct for this nature of the species, the likelihood that identical multilocus genotypes (MLGs) emanate from sexual reproductive events (P_{sex}) should be determined (Arnaud-Haond et al., 2007). The decision that ramets are possibly accounted for by sexual reproduction was made if $P_{sex} > 0.05$ or $P_{sex} > P_{gen}$ (Parks & Werth, 1993). Clonal diversity was measured by four parameters using GENCLONE 2.0 (Arnaud-Haond & Belkhir, 2007). Clonal richness (R) was estimated from the number of distinct MLGs (G) and the number of ramets (shoots) (N) as $R = (G - 1)/(N - 1)$ (Dorken & Eckert, 2001). We calculated

genotypic evenness (ED) to assess equitability in the distribution of MLGs and Simpson's diversity index D. The Pareto distribution index (β), a continuous estimate of the cumulative frequency of the genets containing a particular number of ramets, was used as a proxy for clonal diversity. The distribution follows a power function: $N \geq X = aX^{-\beta}$, where $N \geq X$ is the number of genets containing X, or more, ramets (Arnaud-Haond et al., 2007). The β takes a value from zero to infinity. Higher β value is achieved by higher genotypic richness and evenness of dispersion of ramets among genets (Arnaud-Haond et al., 2007). The spatial pattern of clonemates or MLGs belonging to identical multilocus lineages along 90–145 m transects was determined using an aggregation index (Ac) as $Ac = (P_{sg} - P_{sp})/P_{sg}$, with P_{sg} being the average likelihood of clonal identity of all sample unit pairs and P_{sp} the average probability of clonal identity among pairwise nearest neighbors. This index ranges from zero (when the probability between nearest does not differ) to one (when the probability between nearest neighbors differ in a situation of spatially distant distinct clonal lineages).

Genetic diversity within populations

To reduce the effect of clonal reproduction on allele frequency at each locus due to recounting of the matching genotype, analysis was only conducted for distinct MLGs. As a result, 202 MLGs were used to compute the following genetic diversity parameters for each population: mean number of alleles (A); effective number of alleles (A_e); number of private alleles (PA); deviation from Hardy–Weinberg equilibrium; and observed (H_o) and expected (H_e) heterozygosity with GENALEX 6.5, 3b (Peakall & Smouse, 2006). Linkage disequilibrium was computed using FSTAT and the P value for genotypic disequilibrium was based on 1,000 permutations. Allelic richness (A_r) after correcting for unequal sample sizes and inbreeding coefficient (F_{IS}) for all populations per locus were obtained from FSTAT 2.9.3.2 (Goudet, 2001). Following the assumption of the two-phased and 95% stepwise mutation models, evidence of recent bottleneck events was assessed by applying the Sign test in BOTTLENECK (Cornuet & Luikart, 1996) at 10,000 iterations.

Genetic structure and connectivity

Analysis of molecular variance (AMOVA) was performed using GENALEX to assess the relative partitioning of total genetic variation into within and among individuals and among populations at 999 permutations. Historical gene flow (N_m) was estimated using the F_{ST} method: $F_{ST} = 1/(1 + 4N_m)$ (Slatkin & Barton, 1989). Pairwise genetic differentiation among populations was quantified in terms of F_{ST} and D_{EST}. F_{ST} was computed and the significance level was tested by randomizing MLGs between pairs of populations using FSTAT V.2.9.3 (Goudet, 2001). D_{EST} for each population was calculated using SMOGD v1.2.5 (Crawford, 2010) at 1000 bootstrap replicates. Mantel test (Mantel, 1967) for isolation-by-distance (IBD) was carried out by associating the pairwise values of genetic differentiation (F_{ST}) among populations (Rousset, 1997; $F_{ST}/(1 - F_{ST})$) to distance matrices (log scale) derived from the lake-water current model (Dargahi & Setegn, 2011) (Supplementary Table 3) and the direct flight distance at 999 permutations with GENALEX. Similarly, the IBD was also established by regressing the distance with another measure of allelic differentiation (D_{EST}).

Principal coordinates analysis (PCoA) based on a matrix of pairwise genetic distances between individuals was also carried out. To infer the number of spatial genetic clusters, we applied a Bayesian modeling approach implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2000). The run proceeded with assumptions of admixed ancestry and correlated allele frequencies among populations under 100,000 burn-in periods followed by 500,000 Markov chain Monte Carlo (MCMC) repetitions. The number of clusters (K) was set to 1–6. Each K was repeated 10 times to ensure convergence. The paramount number of genetic clusters was estimated from the saturation value of the mean log-likelihood (Ln P(X|K)) and by the maximum ad hoc quantity, delta K (ΔK), using STRUCTURE HARVESTER (Pritchard et al., 2000; Evanno et al., 2005). The spatial genetic structure estimated using STRUCTURE was visualized with the online program CLUMPAK (Kopelman et al., 2015). The STRUCTURE output was compared with a surface flow model developed by Dargahi and Setegn (2011) so as to verify whether or not the genetic structure follows the main pattern of surface water flow.

Estimating contemporary migration and clonal dispersal

Contemporary migration rate (M_C) and direction among *C. papyrus* populations within the lake were estimated using a MLG-based Bayesian approach with BAYESASS 1.3 (Wilson & Rannala, 2003). Runs were carried out following 3,000,000 MCMC iterations, 1,000,000 burn-in periods, a 2,000 sampling frequency, and with 0.15 as a default delta value of allele frequency. In this approach, inference of migration rates does not depend on the population genetic equilibrium. To detect first-generation immigrants, an assignment method executed in GENECLASS 2 version 2.0 (Piry et al., 2004) was employed by running Monte Carlo resampling (1,000 permutations) to a threshold probability of 0.001 following Paetkau et al. (2004). Subsequently, we employed self-assignment tests to detect the most likely source populations of the immigrant individuals identified from the pool of the sampled individuals, using the assignment criterion suggested by Rannala and Mountain (1997). Clonal dispersal (vegetative spread) was indirectly inferred by identifying individuals sharing the same MLG among populations using a multilocus match function in GENALEX.

Results

Clonal and genetic diversity

Among the 402 ramets analyzed, 202 distinct multilocus genotypes (MLG) were present (50.3%). The remaining 200 individuals (49.7%) were found to have a significant probability of not being separated from each other by a sexual event ($P_{gen} > P_{sex}$). Clonal diversity varied among populations, and it ranged from swamps where almost each ramet represented a distinct MLG (SG; $G = 35$) to a few genets or nearly monoclonal (TG; $G = 3$) (Table 1). Overall, the mean clonal richness (R) and evenness (ED) of *C. papyrus* spanning swamps fringing Lake Tana were 0.50 and 0.64, respectively. The highest degree of clonal diversity was recorded at LA and LY ($R = 0.90$; $ED = 0.99$), which each had 27 genets out of the 30 ramets sampled. In agreement with the R and ED, the Pareto descriptor (β) ranged between 1.13 and 4.91. In

each site, clones were randomly aggregated (AC = 0.01–0.60), reflecting variation in clonal composition due to either phalanx or guerrilla growth forms. Along a linear transect, clones extended from three (at the shortest distance) to 72 m.

Only populations that have more than 12 distinct MLGs were considered for an overall genetic diversity analysis (i.e., excluding TG, and pooling ST and TD, and TB and TW). However, we used all the 202 MLGs for the individual-based analysis (PCoA, STRUCTURE). The remaining 199 unique MLGs contained 95 alleles (3–11 per locus) over 15 microsatellite loci. The MICRO-CHECKER analysis showed no evidence of a scoring error due to stuttering or large allele dropout and null alleles. No linkage disequilibrium was detected between loci. The number of alleles (A) ranged from 1.9 to 3.6, while the effective number of alleles (A_e) ranged from 1.5 to 2.1 per population (Table 1). The mean allelic richness (A_r) per population, accounting for a minimum number of genets ($G = 13$), was about 2.8. In total, 31 private alleles were detected within 10 populations. Population SG harbored the highest number of private alleles ($A_p = 13$ alleles). Populations revealed a moderate overall level of heterozygosity ($H_E = 0.37$; $H_O = 0.36$). Contrary to our expectation, populations located at the outlet (ST and TD) exhibited the lowest genetic diversity. The average inbreeding coefficient was very low ($F_{IS} = 0.01$), although seven populations exhibited positive F_{IS} values that indicate heterozygote deficits. Under both the stepwise mutation and two-phase models, a significant bottleneck signal was detected in four of the 13 populations (ST, TQ, DK, and TB; sign test; $P < 0.05$).

Population genetic structure and connectivity

Hierarchical AMOVA showed that 85% of the genetic variation resided within *C. papyrus* individuals in the total population ($F_{IT} = 0.141$; $P < 0.001$), whereas 5% resided among individuals of populations ($F_{IS} = 0.054$; $P = 0.004$) (Supplementary Table 4). The remaining 10% accounted for the variance among populations ($F_{ST} = 0.10$; 95% CI 0.076–0.116; $D_{EST} = 0.099$; 95% CI 0.002–0.421), with a moderate level of historical gene flow ($N_m = 2.5$). The standardized pairwise genetic differentiation between populations is detailed in Table 2. Pairwise F'_{ST} and

D_{EST} ranged from low ($F'_{ST} = 0.042$; $D_{EST} = 0.0014$; LA vs. LY) to high differentiation ($F'_{ST} = 0.403$; $D_{EST} = 0.121$; TQ vs. TB). Substantially low genetic divergence was observed between neighboring populations (2.5–5 km) in western parts of Lake Tana (LA, LY, and SG). In contrast, some geographically close populations (LM vs. ST) revealed significant genetic differentiation, most likely due to the smaller number of genets (e.g., $N = 13$). We found a weak but significant relationship between allelic differentiation (D_{EST}) and surface flow distance (Mantel test, $R^2 = 0.25$, $P < 0.05$; Fig. 2). Similarly, there was a significant isolation-by-distance ($R^2 = 0.44$, $P < 0.001$) in terms of genetic differentiation ($F_{ST}/(1 - F_{ST})$) against distance along the common surface water flow pattern (Supporting Fig. 1). Within the lake, *C. papyrus* populations genetically differentiated after 10 km, where the pairwise genetic differentiation is higher than 0.213. Neither F_{ST} nor D_{EST} correlated with the direct flight distance.

Based on the assumption of admixture and correlated allele frequency models, the assignment of individuals using STRUCTURE showed three genetic clusters ($K = 3$; Fig. 1a, b) of papyrus populations that have the optimal values of Delta $K (\Delta K) = 268.53$ and Ln $P (K) = -4296.43$ (Supporting Fig. 2a, b). Eighty-nine individuals from five populations (TG, TQ, LY, LA, and SG) were assigned to the first cluster with ancestry membership coefficient (Q) > 80%. Three populations (LR, TW, and LM) located on the opposite shorelines of the lake belonged to one genetic assembly (cluster-II), with admixed individuals (Q > 80%) that were from two gene pools being found together in the same population. The third genetic cluster comprised five populations (TB, DK, TD, ST, and LI) along the path of the Blue Nile River in Lake Tana (except LI) up to the point where bifurcation occurred around the outlet (TD). Most individuals ($N = 66$) in this cluster had Q values above 80%; however, the connection with the other populations is still apparent due to the occurrence of a fraction of mixed individuals. The result of the STRUCTURE analysis is further corroborated by the principal coordinate analysis (PCoA) at the population level. At a population level, the first two axes of the PCoA explained 35.8 and 22.4% of the total variance in allele frequencies, respectively (Fig. 3). In addition, the PCoA at individual level provides an

Table 2 Pairwise comparisons of population genetic differentiation-based F'_{ST} (below diagonal) and D_{EST} (above diagonal) of *C. papyrus*

	TQ	LR	ST[a]	LM	LI	LY	LA	SG	DK	TB[a]
TQ	–	0.0285	0.0543	0.0572	0.0317	0.0198	0.0184	0.0245	0.0177	0.121
LR	0.227***	–	0.0297	0.0422	0.0196	0.011	0.0074	0.0132	0.0222	0.0639
ST[a]	0.381***	0.266*	–	0.054	0.0112	0.0502	0.0241	0.0359	0.0057	0.0421
LM	0.21***	0.164**	0.264*	–	0.0114	0.0407	0.0376	0.0519	0.0282	0.0366
LI	0.164***	0.112[ns]	0.078[ns]	0.076[ns]	–	0.0496	0.0258	0.0403	0.003	0.0279
LY	0.145**	0.15*	0.267[ns]	0.192[ns]	0.177***	–	0.0014	0.0141	0.0308	0.1072
LA	0.139**	0.058[ns]	0.215*	0.164*	0.107***	0.042[ns]	–	0.0024	0.0144	0.0745
SG	0.156***	0.11***	0.289**	0.186**	0.18***	0.099***	0.043**	–	0.0193	0.0777
DK	0.168***	0.141**	0.084[ns]	0.166**	0.037[ns]	0.163***	0.098***	0.133***	–	0.0386
TB[a]	0.403***	0.214[ns]	0.23[ns]	0.17[ns]	0.146[ns]	0.297*	0.225[ns]	0.29**	0.253***	–

Two populations southern tip and Debre Mariam, Upper Blue Nile Womberya were pooled as well as one population with 3 MLGs was not included). See Table 1 for population abbreviations

ns non-significant

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

[a]Pooled
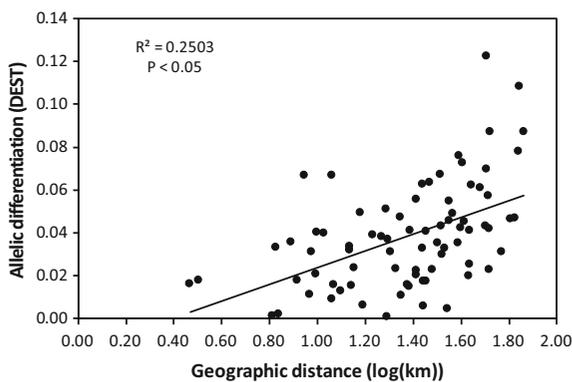
Fig. 2 Relationship between pairwise genetic distance measured as D_{EST} and geographical distance following surface current for *C. papyrus* populations

undifferentiated pattern, although there are 14 genets as an outlier group (Supporting Fig. 3), which were identified as first-generation immigrants using GENECLASS. The genetic structure, PCoA, and migration pattern partly followed the common surface circulation.

Contemporary migration and clonal dispersal

Inter-population contemporary migration rates (Mc) ranged from 0.001 (from ST to TB) to 0.135 (from TQ to LY). In most cases, the Mc between populations demonstrated a bidirectional and asymmetrical pattern (except from LA to LY and LY to SG) dominantly towards populations in south and southwestern Tana (Table 3). According to the assignment tests on the M_C, the likelihood of an individual not being an immigrant to the population where they had been sampled ranged from 0.833 to 0.992. Fourteen *C. papyrus* individuals were identified as first-generation putative immigrants, exhibiting a probability of less than 0.01 for the sampled population using GENECLASS 2 (data not shown). Seven of these immigrants originated from unsampled swamps, since these individuals could not be explicitly assigned to a source population given all the observed allele frequencies.

Clonal dispersal (dispersal via vegetative fragments) was inferred by identifying mutual MLGs shared between/among populations. Between sites, one to four individuals sharing identical MLGs could be identified (Fig. 4). The distances between populations with the same genotypes ranged from 2.5 to 75 km. Additionally, three populations shared two
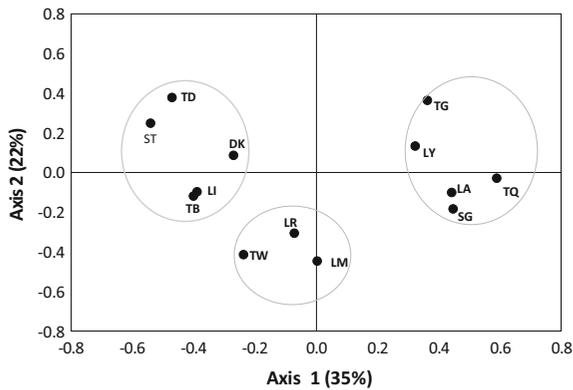
Fig. 3 PCoA grouping of 13 *C. papyrus* populations from Lake Tana using 15 microsatellite loci

individuals with identical MLGs over a distance of 78 km (from TG to DK then to ST), which highlights the potential for dispersal by vegetative fragments or by drifting *C. papyrus* swamps.

Discussion

The application of novel microsatellite markers enabled us to investigate the genetic and clonal diversity, genetic structure, differentiation, contemporary migration, and vegetative dispersal of hydrologically connected *C. papyrus* populations in a dynamic freshwater lake. Some *C. papyrus*

populations showed signs of genetic bottlenecks. However, the papyrus sustained a moderate overall level of genetic and clonal diversity in Lake Tana. This study adds to a growing body of literature reporting long-distance vegetative dispersal in emergent macrophytes (Fer & Hroudova, 2009). The genetic structure, asymmetric contemporary migration rate, and vegetative dispersal mirrored the surface flow and circulation pattern that determine within-lake genetic differentiation. However, our results only present a relation inferred from the isolation-by-distance; thus, further studies are needed to establish causality.

Moderate genotypic and genetic diversity

Similar to several aquatic plants with mixed reproduction modes (Ohsako, 2010; Serra et al., 2010), the *C. papyrus* populations in Lake Tana were composed of genets ranging from monoclonal to multiclonal. Alternatively, this suggests that the populations vary spatially from having no measurable sexual reproduction to having detectable sexual reproduction. Such variation in genet distribution among populations could be a function of environmental factors that trigger a shift in clonal growth and seedling recruitment strategies, as well as a mating system and dispersal mechanisms (van Dijk & van Tussenbroek, 2010; Arnaud-Haond et al., 2012) that ultimately determine clonal richness. In agreement with this

Table 3 Rate of recent migration between 10 *C. papyrus* populations

	TQ	LR	ST[a]	LM	LI	LY	LA	SG	DK	TB[a]
TQ	–	*0.013*	0.008	0.012	0.008	*0.135*	*0.125*	*0.103*	*0.022*	0.007
LR	0.007	–	*0.016*	*0.029*	0.009	0.004	0.01	0.009	0.007	0.01
ST[a]	0.006	0.01	–	*0.017*	*0.014*	0.003	0.003	0.003	0.006	0.001
LM	0.007	0.008	0.008	–	0.004	0.004	0.003	0.003	0.007	0.007
LI	0.007	0.004	0.004	*0.033*	–	0.003	0.003	0.003	0.005	0.004
LY	0.003	0.009	*0.015*	0.007	0.004	–	*0.015*	0.003	0.007	0.003
LA	0.005	0.016	*0.016*	0.008	0.004	*0.015*	–	*0.013*	0.007	0.003
SG	0.003	0.003	*0.016*	0.008	0.004	0.003	*0.013*	–	0.007	0.003
DK	*0.015*	*0.123*	*0.129*	0.009	0.007	*0.011*	0.013	0.011	–	0.007
TB[a]	0.008	0.009	*0.075*	0.009	0.009	0.01	0.009	0.009	*0.058*	–

Values that contain sufficient information to suitably estimate migration relative to 95% confidence interval (mean = 0.0127; CI 1.0×10^{-4} to 0.184) are highlighted in italics. Migration from one population to the other (below diagonal values) and migration from the opposite direction are indicated in the above diagonal

[a]The pooled populations

Fig. 4 Long-distance vegetative dispersal inferred from the number of individuals with identical multilocus genotypes shared between populations and within a population. The largest proportion in each chart represents the number of individuals sharing identical MLGs within a population
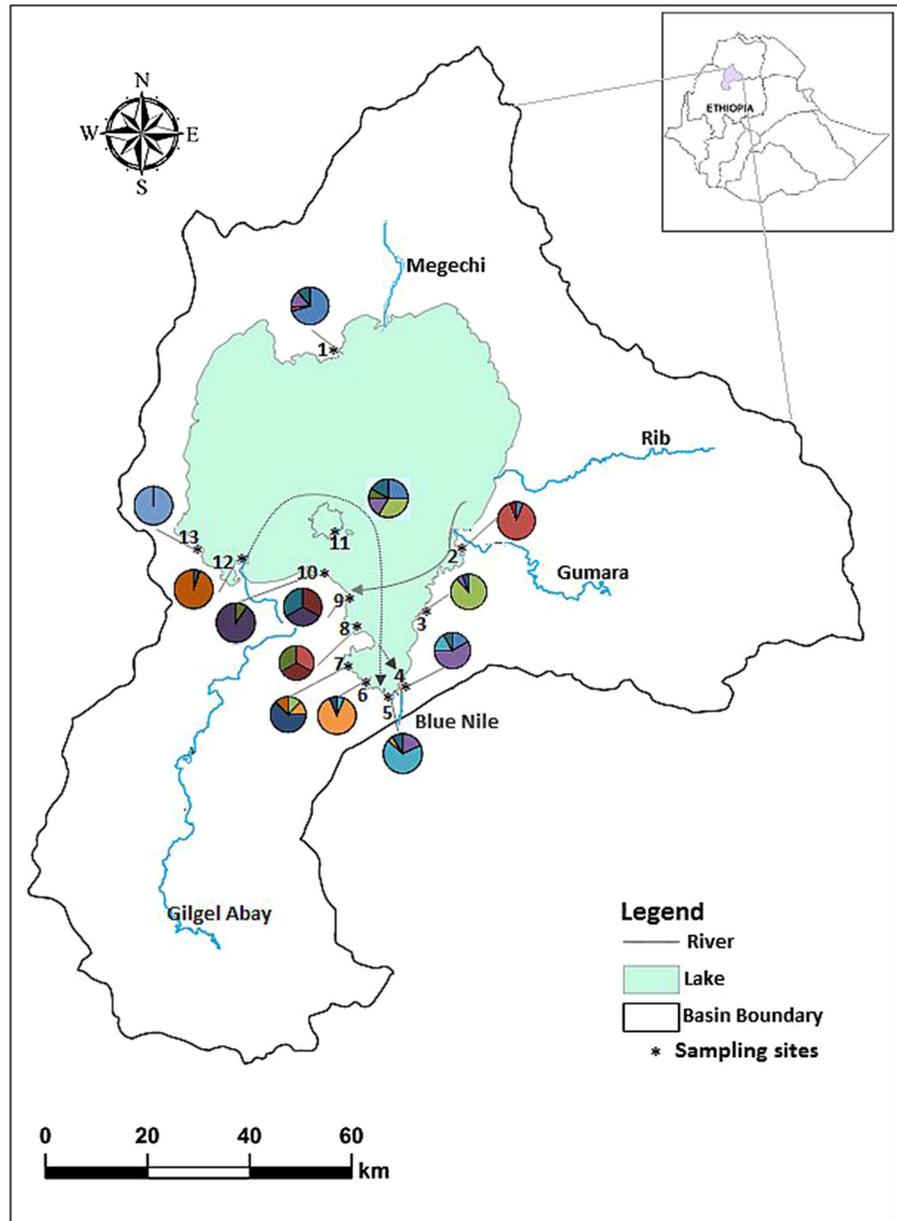
conjecture, mixed clonal growth (phalanx and guerrilla) and seedling recruitment (initial and repeated seedling recruitment) strategies have been reported for papyrus populations from the Rift Valley Lakes, Lake Victoria, and the isolated wetlands of Kenya (Terer et al., 2015). Consequently, the high genotypic richness and evenness of *C. papyrus* populations LY, SG, and LA could be explained by sexual recruitment from seeds (Terer et al., 2014) and input of genets via seed dispersal following the east to southwest surface

flow. In contrast, the low genotypic diversity of the southern Tana populations (e.g., ST) suggests the persistence of a few long-lived clones. In such populations, nearly all individuals belonged to one genet, showing that clones prolifically extend within swamps to distances of hundreds of meters. In addition, a nearly monoclonal population (e.g., TG) probably established from a few seeds of other source populations, perhaps via bird- and human-mediated dispersal and through hydrochoric migration along the

surface flow. Furthermore, the lowest aggregation index in this population infers the recurrent domination of a few clones. The mean level of clonal richness of *C. papyrus* ($R = 0.50$) observed in Lake Tana is considerably higher than populations of several emergent macrophytes, such as *Carex kobomugi* (Ohsako, 2010), *Phragmites australis* (Engloner et al., 2010; Paul et al., 2011), *Typha latifolia* (Tsyusko et al., 2005), and *Sagittaria natans* (Yue et al., 2011). Moreover, the clonal heterogeneity ($\beta = 1.13–4.91$) is higher than the mean values for populations belonging to the 11 clonal plant species compiled by Ohsako (2010; $\beta = 0.930$). This value is also comparable to *C. papyrus* populations of the Rift Valley Lakes, Lake Victoria, and the isolated wetlands of Kenya ($\beta = 1.04–3.90$; Terer et al., 2015).

The overall genetic diversity of the *C. papyrus* populations in Lake Tana ($H_E = 0.37$) is low when compared to the populations in Lake Naivasha ($H_E = 0.55$; Triest et al., 2013) and Lake Victoria ($H_E = 0.644$; Terer et al., 2014). On the one hand, the level of genetic diversity in the present study is higher than in other clonal emergent macrophytes, such as *T. latifolia* ($H_E = 0.29$; Tsyusko et al., 2005), *P. australis* ($H_E = 0.14$; Paul et al., 2011), and *S. natans* ($H_E = 0.28$; Yue et al., 2011). Spatial variation in genetic diversity was observed among the studied populations. Despite this discrepancy, the moderate overall genetic diversity might be due to the mixed reproduction systems of papyrus (Chen et al., 2009; Terer et al., 2014) and the intermediate level of gene flow counteracting genetic drift. In particular, a large number of private alleles (P_A) elevated the genetic diversity of the populations of southwest Tana (SG and LY). In contrast, the small (< 1 ha) populations (e.g., LA and LR) also maintained high genetic diversity levels without harboring private alleles as a result of historical gene flow (Tian et al., 2012). Besides the local genetic bottlenecks promoting genetic drift, prolific vegetative propagation might also play a critical role in the low genetic diversity observed in the south Tana populations.

Seed dispersal by water (hydrochory) is one of the efficient mechanisms that amplify aquatic plants' genetic diversity, particularly in the downstream (Pollux et al., 2009; Schleuning et al., 2011). In contrast to our expectation of high genetic diversity, the low genetic diversity of a population at the outlet may be due to several factors. The first factor could be the prominent disturbances caused by harvesting, habitat destruction due to urbanization or settlement, agricultural expansion, sand mining, and spread of invasive weed species (Wondie, 2010; Minale & Rao, 2012), which limit seedling recruitment (continuous cutting of umbels prior to seed formation negatively influences the seed bank), and fragmentation that leads to population bottlenecks as detected in southern Tana (ST). Second, the dispersal and migration to the outlet might perhaps be relatively high, although the successful immigrant recruitment to the established populations might be low due to selection acting against immigrants (e.g., limited space and habitat for the seedling establishment). This is further reflected in the lower clonal diversity characterized by a few large clones at the outlet. Such large clones are presumably a result of clonal selection that enhances a decrease in genetic diversity (Alvarez et al., 2005). Alternatively, clones that have newly arrived via drifting might compete with existing genets and be favored over sexual recruitment, which would consequently lessen genetic diversity. Third, the high surface flow velocity regulated by wire around the outlet might reduce the retention of migrants or locally produced seeds into a seed bank. Locally reigning hydrodynamic conditions and flow regulation impede the potential for sexual reproduction, water-mediated dispersal, and the recruitment of aquatic plants (Kudoh et al., 2006; Pollux et al., 2007), thereby shaping the populations genetic diversity and structure (Werth et al., 2014). On the contrary, low flow velocity would allow plants to emerge from the water and reproduce sexually, leading to high genotypic diversity at the population level (Pollux et al., 2009).

Genetic structure and differentiation

Interaction of wind and secondary flow controls the mixing processes of Lake Tana (Dargahi & Setegn, 2011). In the case of a fully hydrological mixing system that facilitates hydrochory, one assumes a uniform genetic pattern. However, the Bayesian-based structure analysis revealed three non-panmictic clusters of *C. papyrus* populations following the surface water flow pattern. Within-lake genetic differentiation revealed a weak but significant IBD when regressed with distance along the surface flow. The weak IBD suggests that the IBD alone may not explain the obtained genetic structure and differentiation. In the

case of Lake Tana (Dargahi & Setegn, 2011), geographic distance alone could not always sufficiently infer gene flow, particularly when hydrochoric dispersal takes place along an asymmetric circulation pattern (Wee et al., 2014). In addition to the moderate historical gene flow ($N_m = 2.5$), an asymmetrical recent migration rate and long-distance clonal dispersal likely represent a plausible reason for the weak IBD. Despite *C. papyrus* populations becoming spatially structured after about 10 km ($F_{ST} = 0.21$), their connectivity remains apparent from clones that are dispersed over 75 km. In contrast to the gene flow among populations of linearly arranged riparian landscapes (Triest et al., 2010; Mitsui et al., 2010), here it is not constrained to neighboring populations surrounding the lake system. Aggregation of populations (e.g., LR, TW, and LM) located on the opposite shorelines to the same genetic cluster suggests that hydrological connectivity may facilitate the influx of alleles. In many aquatic systems, seed dispersal occurs over tens of kilometers (Morris et al., 2011) and hence upholds population genetic connectivity. On the contrary, high genetic differentiation, low contemporary migration, and a deficit of vegetative dispersal among adjoining populations (e.g., LI and LY) show asymmetric circulation around the shore, the Zege peninsula, and the two islands (Kibran and Entos), which could act as physical barriers to gene flow. Nevertheless, the overall pattern of the genetic structure follows the surface flow from north to south and from east to southwest Tana (Dargahi & Setegn, 2011), pointing a stochastic mixture of MLGs ensuing from water-mediated seed and vegetative dispersal via the drifting of swamps. In addition to the surface circulation patterns, mating system, mode of dispersal (Ohsako, 2010), and extent of wetland fragmentation (Wondie, 2010) may also have affected the genetic differentiation and structure of *C. papyrus* populations. Such an effect could be indirectly inferred from spatial variations in clonal richness, allelic diversity, and inbreeding (F_{IS}) among populations. For example, the wide range of clonal aggregation index highlights the shift in clonal growth strategies as a response to local factors that determine the size of ramet patches and genetic differentiation (Lembicz et al., 2011). The degree of fragmentation of each swamp may also contribute to the contrasting genetic differentiation between populations. The clumping of three neighboring populations (LY, SG, and LA) of southwestern

Tana into one genetic cluster corresponds with recent fragmentation of a formerly large swamp due to prominent human encroachments (Wondie, 2010). Although these populations appear isolated (albeit only 2.5 km apart) and patchy, the frequencies of common alleles indicate that either wetland destruction occurred recently or the alleles are shared due to migration.

Asymmetrical contemporary migration

Except for symmetrical migration between spatially proximate populations (LA to LY and LY to SG), recent migration generally follows a bidirectional asymmetrical pattern biased toward south and southwest Tana. Strong southward surface flow, weak clockwise circulation, and impasse caused by several major and minor islands within the lake may explain the asymmetric migration. In addition, bottlenecked populations might have low outgoing migration (e.g., TQ and ST) to the non-bottlenecked ones, thereby causing the asymmetrical pattern. Asymmetric migration is common while propagules undergo long-distance dispersal (LDD) through wind or water (Paz-Vinas et al., 2013). In instances where drawdown is prominent, wind alone might disperse papyrus seeds; however, due to its random nature, establishing a causal relationship with the migration pattern and genetic structure at the lake scale remains challenging. Although the seeds of *C. papyrus* are primarily dispersed by wind (Terer et al., 2012) at local scale, the asymmetric migration identified in the present study suggests that hydrochory mainly contributes to sustaining both the genetic structure and connectivity among populations. The asymmetric migration pattern could be attributed to transport of seeds by humans via papyrus-made boats, locally 'tanqua' during fishing, papyrus harvesting and random movement for daily activities across the lake. Humans dispersed seeds unintentionally when moving through the landscape/ seascape by motor vehicles or boats (Coutts & Dodgshun, 2007; Donnelly & Walters, 2008). High contemporary migration rates ($M_C > 0.1$) between populations (e.g., TQ, LY, LA, and SG) that coincide with the wind-driven surface flow pattern (Dargahi & Setegn, 2011) from east to southwest Tana (226.7°) reflect the role of hydrological connectivity in population genetic connectivity. The effect of water currents on the genetic structure and migration pattern

is a common phenomenon in aquatic plants, including seagrasses (Serra et al. 2010; Ruiz-Montoya et al., 2015; McMahon et al., 2015), mangroves (Wee et al., 2014; Ceron-Souza et al., 2015), and intertidal alga (Muhlin et al., 2008). Moreover, tracing the source population of immigrants has an important implication for a better understanding of the within-lake connectivity pattern. Despite the fact that the existing *C. papyrus* swamps around the lake margin were sampled, the assignment test detected seven individuals as first-generation immigrants from another source population. These immigrant individuals could be either remnants of locally destroyed populations or novel genets from the upstream (Gilgel Abay River).

Clonal dispersal

In aquatic clonal plants, the drifting of either loose-lying rhizomes or detached swamps with live plants represents an alternative means of dispersion (Sarneel, 2013). Detached papyrus swamp or rhizome fragments may move due to hydrological dynamics and human pressures during harvesting and hence establish new floating islands (Azzaa et al., 2002). However, such cases seem to be uncommon from the perspective of significant overall genetic differentiation because in most instances, these phenomena promote genetic connectivity among distant sites (Ronce, 2007). The occurrence of two to four individuals with identical MLGs among swamps implies clonal dispersal through detached rhizomes and drifting vegetative mats. Individuals with identical MLGs at populations approximately 78 km apart suggested the long-distance vegetative propagule dispersal of *C. papyrus*. Water-mediated inter-population clonal dispersal of propagules drives migration (Nilsson et al., 2010), enhances gene flow (Ronce, 2007), and alters population dynamics (Cousens et al., 2008). Although the distance to disperse varies from species to species, similarly effective LDD of vegetative propagules and colonization events has been accounted for different aquatic plants *Nuphar lutea* (Fer & Hroudova, 2008), *Vallisneria spinulosa* (Chen et al., 2007), *Potamogeton pectinatus* (Triest & Fenart, 2014), *Zostera noltii* (Berkovic et al., 2014), and *Phragmites australis* (Fer & Hroudova, 2009), different submerged taxa (Triest et al., 2010), and riparian fen species (Sarneel, 2013). In agreement with the contemporary migration, the clonal dispersal follows

the flow gradient and circulation direction towards the populations of the outlet (southern Tana) and south-western Tana, respectively. Such a dispersal of vegetative fragments and floating seeds in large but shallow freshwater lakes (e.g., Lake Tana) is likely to rely on stochastic hydrological forces and wind pressure. Unlike floating seeds, which are most likely evacuated through the outlet, floating mats can be retained near the outlet.

Conservation implications

Maintenance of high levels of genetic diversity through gene flow or connectivity is one of the key elements of ecosystem restoration and species conservation (Frankham et al., 2002; Reynolds et al., 2012), as its loss significantly restrain adaptability to dynamic and continually changing environments (Hoffmann & Willi, 2008). Accounting the ecological regulatory and provisional services that *C. papyrus* provides in wetlands and to the surrounding communities (Owino & Ryan, 2007; Terer et al., 2012) measures to sustain resilience are critical. Regardless of the remarkable spatial genetic difference among populations, the overall moderate genetic and genotypic diversity partly highlight the resilience of papyrus swamps. However, populations under anthropogenic disturbance such as TG, TD, TQ, and TB showing signature of recent genetic bottlenecks should be precedence for restoration. Long-distance vegetative propagules dispersal between populations could promote colonization of new sites provided hydrological connectivity is sustained. We suggest that efforts toward conservation and management of papyrus in and fringing Lake Tana should be aimed at sustaining habitat connectivity and population establishment. In addition, for conservation of the studied papyrus swamps, protective measures should be taken to reduce anthropogenic pressures including sand mining, overharvesting, and drainage of swamps for agricultural purposes (Wondie, 2010): these have negative impacts on the substrate, affecting the growth and the recruitment of new genets.

Conclusions

Despite the detected genetic bottleneck, the *C. papyrus* populations in Lake Tana maintained

moderate clonal and genetic diversity with no detectable accumulation at the outlet. There is evidence of long-distance clonal dispersal among populations up to 78 km. The genetic structure, clonal dispersal, and asymmetrical migration partly correspond to the surface flow pattern, although empirical tests to establish the casual relationships are still required. In conclusion, our study highlights the role of hydrological connectivity and vegetative dispersal in shaping the genetic structure, connectivity, and diversity of the *C. papyrus* populations of a dynamic lake. The role of multiple dispersal agents (e.g., wind and water) can be inferred from the asymmetrical migration. Thus, further studies are required to address how far wind disperses *C. papyrus* seeds as well as the extent to which it contributes to the spatial genetic structure within a swamp.

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