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Barriers to genetic connectivity of smooth flatsedge (*Cyperus laevigatus*) among alkaline-saline lakes of Eastern Rift Valley (Kenya)

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ABSTRACT

The saline-alkaline Rift Valley lakes of Kenya are isolated habitats supporting emergent halophytes on the shorelines. *Cyperus laevigatus* L. (Smooth flatsedge) is common to these endorheic lakes suggesting connectivity over long distances. The main objective of this study was to assess the amount and pattern of genetic diversity in *C. laevigatus* populations in wetlands and along shorelines of lakes of the Eastern Rift Valley in Kenya. The clonal, allelic and gene diversity, population genetic structure and fine-scaled spatial genetic structure were assessed on 204 *C. laevigatus* individuals from nine populations, using thirteen newly developed microsatellites. *Cyperus laevigatus* populations maintained high levels of clonal and allelic diversity, though with significant within-population inbreeding. No or only restricted local clonal growth over few metres could be found along shorelines of most lakes. A fine-scaled spatial genetic structure was revealed on sheltered populations indicating contemporary local dispersal from repeated seedling recruitment. Significant differentiation and isolation-by-distance was observed, supporting a stepping-stone model. A north to south gradient, as revealed from pairwise F_{ST}, PCoA, Structure and a Barrier analysis, included barriers between some lakes, with Lake Magadi fully separated. Bayesian clustering of individuals revealed a gene pool corresponding to the Great Nakuru-Elementaita basin. Historical hydrological connectivity during Holocene as well as geographical distances between Rift Valley lakes were proposed as major driving forces explaining the contemporary genetic structure.

1. Introduction

Aquatic plants are assumed to have a broader distribution compared to their terrestrial counterparts, despite occurring in island-like habitats, which has been attributed to the uniformity of the aquatic environment, the widespread clonality of aquatic plant species, a balance between modes of reproduction which affect patterns of dispersal and a high phenotypic plasticity (Santamaria, 2002). The balance between sexual and asexual reproduction modes also has been found to have a profound effect on the evolutionary and ecological consequences of dispersal, gene flow, recruitment and the spatial patterns of genetic diversity observed in aquatic plants (Eckert et al., 2016). The extent of fine-scaled spatial genetic structure (FSGS), defined as the non-random spatial distribution of genotypes, is influenced by factors such as the interactions between the temporal processes of vegetative growth and sexual reproduction, species' dispersal ability (Vekemans and Hardy, 2004), natural selection and genetic drift (Curtu et al.,2015; Ohsako, 2010).

At large geographic scales, barriers such as mountain ranges (Abbasi et al., 2016) or isolated lakes and wetlands (Geremew and Triest, 2018; Terer et al., 2015) are confounding factors for the isolation-by-distance (IBD) that can usually be found in aquatic plant populations (Triest et al., 2010, 2018). Connected rivers and wetlands usually allow for vegetative spread of a few genotypes over large distances within the catchment. Also within a single large lake, clonal spread can reach tens of kilometres e.g. *Cyperus papyrus* L. in Lake Tana (Geremew and Triest, 2018). In the case of historical or contemporary gene flow, including long-distance-dispersal (LDD), it can be expected that endorheic lakes would exhibit a common gene pool of a species. In all other scenario's, IBD or barriers can be expected (Terer et al., 2015; Triest et al., 2018).

The endorheic alkaline-saline lakes of the Kenyan Rift Valley are simple in biodiversity with unexpected shifts in species composition due to their highly stochastic environmental dynamics (Schagerl et al., 2015). These alkaline-saline lakes are inhabited by restricted (semi-) aquatic flora and fauna that are highly adapted to the conditions in and around the lakes such as high water temperature, elevated salinity and

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low levels of oxygen (Kipkemboi, 2016; Prinz et al., 2009). These Rift Valley lakes are isolated habitats that support highly adapted emergent halophytes, because the littoral wetlands surrounding these lakes have salinities above 160 mS.cm^{-1} which exert pressure and restrict plant development (Kipkemboi, 2016). The halophytes show broad tolerance to osmotic stress, hydrological changes and physical disturbances from trampling or grazing (Kipkemboi, 2016; Prinz et al., 2009).

Cyperus laevigatus L. is a perennial sedge commonly known as smooth flat-sedge. It has a subcosmopolitan distribution covering the subtropical regions with hot and arid climates together with the tropical, warm temperate regions worldwide, and being widespread throughout Africa (Gupta and Juffe Bignoli, 2013). In Kenya, *C. laevigatus* is very common along the shores of most of the alkaline-saline lakes and wetlands where it is mainly found growing with *Sporobolus spicatus* (Vahl) Kunth. (Onkware, 2000; Ssegawa et al., 2004). The species serves as a major forage source for both domestic and wild animals like buffaloes, especially during the drought periods (Kutilek, 1974; Kipkemboi, 2016). The *C. laevigatus* populations all occur in the dry areas of the Rift Valley and they are the last resort for forage, both for the herbivorous wildlife and pastoralist cattle (especially Maasai community) during the long drought periods and hence, there is a need for the conservation of the marshes in order to support those who utilize them.

The overall objective of this study was to assess the prevailing reproductive strategy, and the amounts and patterns of genetic diversity in *C. laevigatus* populations over the large geographical scale of the Eastern Rift Valley in Kenya. We assumed an absence of clonal spread between endorheic lakes, only within. Specific aims were to (1): estimate the level of clonal and genetic diversity within and among populations in relation to its mixed reproduction mode; (2) test for contemporary fine-scaled spatial genetic structure in *C. laevigatus* within a shoreline; and (3) determine the large-scale genetic structure of *C. laevigatus* populations and test for isolation-by-distance and barriers to infer historical connectivity. For this purpose, novel microsatellite markers first had to be developed for *C. laevigatus*.

2. Materials and methods

2.1. Study sites

A total of 204 individual shoots of *C. laevigatus* were sampled and collected for DNA analysis from eight alkaline-saline lake shorelines and one isolated wetland in Kenya, namely populations from Sadhana Forest site, Lakes Bogoria, Solai, Nakuru, Elementaita, Oloidien, Sonachi and Magadi (Annex: KMZ Google Earth file) between November 2011 and July 2016. They comprised of 13–37 transect samples per location at a minimum distance of 3-5 m between one shoot (ramet) to the other. The geographical distance between pairs of populations ranged from 3.5 to 314 km. Individual shoots were collected from clumps of *C. laevigatus*, kept in paper envelopes and sun dried awaiting DNA extractions.

2.2. Microsatellite development

Prior to application and studying genetic diversity, we developed microsatellite markers using *C. laevigatus* plants of Lake Bogoria (Kenya) as source material. Genomic DNA was extracted from dry and crushed stalks using the E.Z.N.A. SP plant DNA Mini Kit (Omega biotek, Norcross, GA, USA). Purity and quantity of the DNA were determined using a Nanodrop one Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). An Illumina paired-end library was constructed and sequenced using the Illumina HiSeq platform at Macrogen (Seoul, Republic of Korea). SSR_pipeline (Miller et al., 2013) was used to identify microsatellites and design primers. Out of 18.2 million 100 bp paired-end reads, 2.7 million pairs were successfully joined by the module joinseqs. The module SSR_search found 11.607 dinucleotide SSRs with at least 10 repeats, 39.958 trinculeotide SSRs with at least 8 repeats and 3.599 tetranucleotide SSRs with at least 6 repeats. Batchprimer 3 (You et al., 2008) was used for designing primers and 56 primer-pairs were selected for synthesis on the basis of fragment length and the number of repeats. Genomic DNA from 12 randomly selected *C. laevigatus* individuals of other lakes was used for primer testing and PCR products were tested for amplification and polymorphism by capillary electrophoresis on a Qiaxcel (Qiagen). Using Multiplex Manager (Holleley and Geerts, 2009) one single multiplex reaction of 13 amplifiable primer pairs, with 4 different dye-labelled primers (6FAM/VIC/NED/PET), was designed. Fragments were amplified (Ta = 57 °C) using the Qiagen Multiplex PCR Plus Kit (Qiagen). PCR products were run on a ABI3730XL sequencer (Macrogen, Seoul, Republic of Korea) and fragments were scored with GeneMarker V2.20 (Softgenetics LLC).

2.3. DNA extraction and PCR

Genomic DNA extractions were performed on all samples according to the abovementioned method. Thirteen polymorphic microsatellite loci (CLK32, CLK41, CLK12, CLK14, CLK27, CLK39, CLK1, CLK6, CLK45, CLK55, CLK8, CLK42, CLK51) were selected which represented di-, tri- and tetranucleotide repeats (Supplementary Table 1). Multiplex polymerase chain reaction (PCR) conditions for each PCR reaction were: 6.25 µl master mix (Qiagen multiplex pcr kit master mix), 1.25 µl primer mix, 2.5 µl H_2O and 2.5 µl of genomic DNA, making a total volume of 12.5 µl. PCR was performed in a thermal cycler (MJ research PTC-200 and Bio-Rad MyCycler) with the following conditions: an initial denaturation of 95 °C for 15 min followed by 35 cycles of: 30 s denaturation at 95 °C, 90 s annealing at 57 °C and 80 s elongation at 72 °C followed by a final extension of 30 min at 60 °C. PCR products were run on ABI3730XL sequencer (Macrogen, Seoul, Korea) and fragments analysed with GeneMarker V2.60 (SoftGenetics LLC®, State College, USA).

2.4. Data quality check and analysis of genetic diversity

A linkage test between all pairs of loci (1000 permutations) was done across all populations to verify any genotypic disequilibrium at the 0.001 level using FSTAT (v.2.9.3) (Goudet, 2002). MICRO-CHECKER software (Van Oosterhout et al., 2004) was used to verify presence of null alleles, large allele dropout or scoring errors. Transect-based data sets from each population were used for the analysis of clonal and genotypic diversity. Genotypic richness was estimated according to $R = (G - 1)/(N - 1)$, where G is the number of distinct genotypes and N is the sample size. The potential length of genets was estimated from the transect intervals and position of each multilocus genotype (MLG) and their eventual clonal repeats as ramets. After excluding the 41 repeated MLGs, we performed all further analyses and calculated genetic diversity measures on 163 unique genets using GenAlex 6.5 (Peakall and Smouse, 2012) and FSTAT 2.9.3.2 (Goudet, 2002). At population level, we estimated the number of distinct multilocus genotypes (G), genotypic richness (R), mean number of alleles (A_M), effective alleles (Ae), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe) from GenAlex; total number of alleles (A_T), and allelic richness (A_R at k = 16) from FSTAT. The Weir and Cockerham (1984) estimation of F_{IT} (Cap F), F_{IS} (small f) and F_{ST} (θ) for the total population as well as the within-site inbreeding coefficient (F_{IS}) and p-values after randomization tests (1/1000) were obtained from FSTAT.

2.5. Data analysis of genetic structure

A Bayesian clustering analysis of individuals was conducted using STRUCTURE v.2.3.4 (Pritchard et al., 2000) by testing K values ranging from 1 to 10 (with 20 runs per K value). The length of burn-in period

was set at 50,000 and number of Markov chain Monte Carlo repeats (MCMC) after burn-in at 100,000 repeats. The program was run by assuming an admixture model and without prior information on populations. The results of K values were obtained from STRUCTURE HARVESTER online (Earl and vonHoldt, 2012) and the best K value was determined with the highest ΔK value following the Evanno et al. (2005) method and compared to ln (K) convergence.

Genetic structure was assessed using hierarchical analysis of molecular variance (AMOVA) and estimated pairwise genetic differentiation (F_{ST}) for all population pairs using GenAlEx. The regional hierarchy was based on the Bayesian clustering obtained from STRUCTURE for K = 4 with clusters of North (Sadhana Forest site, Lake Bogoria and Lake Solai); Central 'Great Nakuru' (Lake Nakuru, Lake Elementaita and spring); Central 'Naivasha' (Lake Oloidien and Lake Sonachi) and South Rift Valley (Lake Magadi). Isolation-by-distance (IBD) among populations of all saline lakes was estimated through a Mantel test of pairwise genetic differentiation (F_{ST})/(1- F_{ST}) versus geographic distance (log scale) using GenAlEx based on 9999 permutations. A principal coordinate analysis (PCoA) was performed at the individual level based on codominant genotypic distances using GenAlEx. SPAGeDi 1.5a (Hardy and Vekemans, 2002) was used to test for F_{ST} and R_{ST} (1000 permutations) and to test for the slope of the regression over full distance at distance classes of 30, 50, 150 and 315 km. To determine zones of sharp genetic changes across the geographical area, the coordinates and the F_{ST} data matrices were analysed with the Monmonier's algorithm, implemented in Barrier 2.2 software (Manni et al., 2004) and allowed a maximum number of three barriers based on the geographical locations of the sampled populations. To investigate fine scale genetic structure (FSGS) within individual populations, the autocorrelation coefficient (Smouse and Peakall, 1999) for multiallelic codominant markers was used in GenAlEx. Even distance classes of 5, 10, 20 and 30 m of pairwise individual relatedness within each population, thereby excluding the clonal repeats, were considered and based on 1000 permutations. Averaged Kinship values (F_{LJ}) within each population were estimated according to Loiselle et al. (1995) using SPAGeDi.

3. Results

3.1. Clonal and genetic diversity

The thirteen SSR markers showed sufficient resolution power to discriminate all genets for each of the *C. laevigatus* locations with a probability of identity as low as 2.9×10^{-14} to 2.9×10^{-7}. There was no evidence of linkage disequilibrium, null alleles or large allele dropout (size ranges from 57 bp to 214 bp). At the locus level, 8 to 25 alleles were revealed at heterozygosity levels ranging from H_E = 0.405 to 0.812 for the total population (Supplementary Table 1). After excluding the repeated MLGs, 163 out of 204 *C. laevigatus* individuals remained from all nine transects. The mean clonal richness reached R = 0.83 (ranging from 0.52 to 1) and was moderate to very high with six populations having R values of 0.9–1 (Table 1). Genets in linear transects were usually estimated less than or up to 3–5 m long (because of transect design) in most populations, except in Lake Elementaita (average 9 m, ranging from 3 to 25 m), Lake Sonachi (average 3 m, from 3 to 6 m) and Lake Magadi (average 8.1 m; ranging from 3 to 18 m).

A total number of 193 alleles in 13 loci (ranging from 40 to 101 per population) was revealed across the studied region. Allelic richness ranged from 2.5 to 4.9 (mean = 3.9) while the effective number of alleles ranged from 2.2 to 4.6 (mean = 3.4) across the populations (Table 1). Observed heterozygosity ranged from 0.359 to 0.636 (mean = 0.551) while unbiased expected heterozygosity ranged from 0.488 to 0.757 (mean = 0.649). The inbreeding coefficients at the population level ranged from -0.158 up to 0.320 ($p < 0.001$) indicating significant levels of inbreeding in most populations (Table 1). Lake Magadi was the only population that had higher levels of observed than expected heterozygosity. When considering overall inbreeding coefficients (F_{IT} = 0.31) most loci behaved the same within (F_{IS} = 0.16) and between (F_{ST} = 0.18) populations (Table 1), indicating an about equal level of inbreeding within and between populations. A total of 43 private alleles were identified from 12 out of 13 SSR markers. The number of private alleles ranged from 2 in the Sadhana Forest site to 8 from Lake Nakuru, the former being attributed to the small number of genets remaining after repeated MLGs exclusion (Table 1). The frequency of private alleles was highest in Lake Magadi.

Kinship values (F_{LJ}) of *C. laevigatus* populations were highest along the shorelines of Lake Bogoria, Lake Elementaita, Lake Sonachi and Lake Magadi (Table 1). This elevated relatedness allowed detection of fine-scaled spatial autocorrelation of individuals within populations using four distance classes of 5, 10, 20 and 30 m. A significant spatial structure was found again in Lake Bogoria, Lake Elementaita, Lake Sonachi and Lake Magadi within shortest distances of 5 m. At a next distance class up to 10 m, spatial autocorrelation was only marginally significant for Lake Bogoria (Table 1) and absent in all other cases.

3.2. Genetic structure

An analysis of molecular variance (AMOVA) showed about 18% of overall genetic differentiation across the nine *C. laevigatus* populations, whereas 16% could be attributed to differences among individuals within populations. Despite a moderate overall differentiation (F_{ST} = 0.176, $p < 0.001$), pairwise comparisons of genetic differentiation ranged from 0.027 to 0.895 between populations (Table 2). Most population pairs were significantly differentiated, except Lake Nakuru vs. Lake Elementaita SP (F_{ST} = 0.104) and within closest vicinity Lake Oloidien vs. Lake Sonachi (F_{ST} = 0.027). Moderate differentiation (F_{ST} = 0.163 – 0.374) was observed within the central Rift Valley region (Lakes Nakuru, Elementaita, Oloidien and Sonachi) and within the north Rift Valley region (F_{ST} = 0.281 – 0.492). A PCoA at the level of all individuals revealed a gradient along the first axis that explained 14.4%, whereas the second axis explained 7% of the total variation (Fig. 1). Lake Magadi genets appeared as an outlier group which could be attributed to abovementioned elevated frequency of private alleles. *C. laevigatus* populations additionally differentiated on the first axis along a North–South gradient from Lake Bogoria (northern Rift Valley) to Lake Oloidien (central Rift Valley). Most of the individuals from the northern Rift Valley were clearly separated with very little overlap.

Bayesian clustering analysis implemented on STRUCTURE for 163 individuals from all locations assembled these genets into two to four clusters based on $\Delta K = 2$ (Ln P(K) = −7105.8), $\Delta K = 3$ (Ln P(K) = −6698.5) and $\Delta K = 4$ (Ln P(K) = −6480.3) respectively. $\Delta K = 2$ represented a major structure, separating Lake Magadi. The $\Delta K = 3$ showed moderate admixture of populations from Lake Bogoria, the Sadhana Forest site and Lake Solai, all located in the northern region. No admixture was found between the populations of Lakes Nakuru, Elementaita, Oloidien and Sonachi which represented the Central Rift region. $\Delta K = 4$ additionally subdivided the Central Rift region, namely Lake Nakuru and Lake Elementaita against distantly located smaller crater lakes (Lake Oloidien and Lake Sonachi). Consequently, both at $\Delta K = 3$ and $\Delta K = 4$, Lake Magadi remained a fully separated cluster without admixture (Fig. 2).

An hierarchical AMOVA consisting of abovementioned three or four regional clusters (Table 3) had almost similar F_{RT} values (0.145 and 0.140 respectively; $p < 0.001$). When compared with the F_{ST} values of these three and four regions (0.219 and 0.189 respectively), a hierarchical AMOVA indicated substantial contribution of both the level of regions and the level of lake populations to the overall differentiation. A Mantel test revealed isolation-by-distance (R^2 = 0.40, p = 0.038) considering all nine *C. laevigatus* populations (Fig. 3). A further testing of given distance classes revealed significantly lower differentiation (F_{ST} = 0.07, p = 0.008) than average (F_{ST} = 0,163) only for populations within less than 30 km. Populations within a distance class above

Table 1
Clonal and genetic diversity descriptive statistics over 13 nuclear microsatellite loci for 9 *C. laevigatus* locations: N, number of ramets; G, number of genets; R, clonal richness; L, estimated average length of ramets (in meter); A_T, total number of alleles; A_M, mean number of alleles; A_E effective number of alleles; A_R, allelic richness; A_P, number of private alleles per population; H_O, observed proportion of heterozygotes; uH_E, unbiased proportion of heterozygotes; F_{IS}, inbreeding coefficient; F_{LJ}, kinship value relative to whole population; FSGS, fine-scaled genetic structure presence at given distance class and significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{NS}, non-significant.

Locality	Lat	Long	N	G	R	L	A_T	A_M	A_E	A_R	A_P	H_O	uH_E	F_{IS}	F_{LJ}	FSGS
Sadhana Forest site	0.8627	36.8089	13	8	0.58	< 3	65	4.5	3.8	4.5	2	0.636	0.751	0.167***	0.077	NS
Lake Bogoria	0.3065	36.0768	25	23	0.92	< 5	95	4.7	4.5	4.7	7	0.629	0.744	0.157***	0.154	5m***-10m*
Lake Solai	0.0611	36.1672	19	19	1.0	< 5	101	4.9	4.6	4.9	7	0.626	0.757	0.178***	0.080	NS
Lake Nakuru	−0.3230	36.0874	14	14	1.0	< 5	85	4.2	3.2	4.2	8	0.537	0.681	0.217***	0.071	NS
Lake Elementaita Spring	−0.4726	36.2583	10	10	1.0	< 5	65	3.9	3.3	3.9	3	0.587	0.642	0.092**	0.116	NS
Lake Elementaita	−0.4370	36.2560	30	16	0.52	< 9	47	2.9	2.5	2.9	2	0.359	0.522	0.320***	0.214	5m***
Lake Oloidien	−0.8255	36.2775	25	25	1.0	< 5	92	3.9	3.0	3.9	6	0.532	0.630	0.160***	0.078	NS
Lake Sonachi	−0.7833	36.2600	31	28	0.90	< 3	95	3.9	3.0	3.9	4	0.447	0.627	0.291***	0.081	5m**
Lake Magadi	−1.9160	36.3037	37	20	0.53	< 8	40	2.6	2.2	2.5	4	0.603	0.488	−0.158^{NS}	0.511	5m*
Total or mean			204	163	0.83		193	3.9	3.4	3.9	43	0.551	0.649	0.158	0.154	

150 km reached $F_{ST} = 0.24$ and a comparatively higher $R_{ST} = 0.36$. The slope of the regression over full distance was significant for both F_{ST} (log b = 0.07; $R^2 = 0.28$; p = 0.025) and for R_{ST} (log b = 0.12; $R^2 = 0.32$; p = 0.024). This $R_{ST} > F_{ST}$ refers to a larger effect from allele size differences as an evolutionary event over long distances than from allele identity only. A barrier analysis to check for genetic boundaries between neighbouring populations revealed a strong boundary for all 13 loci between Lake Magadi and crater lakes Sonachi and Oloidien (Fig. 4). Populations from Lakes Elementaita and Nakuru showed a second order barrier with crater lakes Sonachi and Oloidien.

4. Discussion

4.1. Clonal strategy

Six out of the nine populations showed a high clonal richness at the transect level which could be attributed to sexual modes of reproduction followed by local vegetative growth, similarly observed in *C. papyrus* (Terer et al., 2015). Ramets usually were less than three metres in size whereas only few shorelines of Lakes Elementaita and Magadi showed clonal extensions of up to 18 and 25 m, respectively. Such very high levels of clonal diversity, suggests frequent seedling recruitment (Triest et al., 2014) which may be attributed to the stressful conditions (Kirk et al., 2011) in which *C. laevigatus* grows. We assume a repeated seedling recruitment (RSR) strategy for *C. laevigatus,* with a continuous recruitment of new genets through seeds which allows survival of young small clones and coexistence of clones of variable age and size, resulting in a high local clonal diversity (Eriksson, 1989; Ohsako, 2010).

4.2. Fine-scaled spatial genetic structure

From the nine populations of *C. laevigatus* populations studied, a weak though significant FSGS was detected at distances less than < 5 m along shorelines of four lakes (Lakes Bogoria, Elementaita, Sonachi and Magadi) which reflects very local contemporary dispersal and recruitment. All of these locations had one thing in common, they were sheltered on the sides by hills or escarpments that may act as wind breaks thereby affecting the wind dispersal of pollen and seeds. Lake Bogoria occupies a narrow half graben escarpment rising to 700 m. Lake Elementaita is flanked by an escarpment and hilly ranges. Lake Sonachi is a small crater lake with a steep catchment of the crater rim of 30–115 m high (Verschuren, 1999) whereas Lake Magadi is flanked by escarpments (Atmaoui and Hollnack, 2003). *Cyperus laevigatus* populations from the remaining saline lakes and wetlands did not reveal a spatial structure in their populations suggesting mixed recruitment of unrelated seeds over various distances along their shorelines. This could be attributed to the basins being more open, namely Lake Solai, Lake Oloidien and the Sadhana Forest site, whereas for Lake Nakuru, dispersal by herbivorous wildlife in the park could be expected, mostly by buffaloes that feed and often rest on the *C. laevigatus* especially during the dry seasons when grasslands dry up (Onkware, 2000). It can be hypothesized that a variety of local dispersal agents for *C. laevigatus* seeds along a shoreline encompasses hydrochory (and anemochory in the case of lowered water level or draw-down) as well as large animals (especially buffaloes and cattle) and foraging waterfowl.

4.3. Barriers and isolation-by-distance

A strong IBD was observed among all pairs of *C. laevigatus* populations, which generally increased over long distances of up to more than 300 km. A similar IBD pattern was found among *C. papyrus* populations from Kenya wetlands up to distances of 565 km (Terer et al., 2015) and clear structure was observed for papyrus wetlands in Ethiopia, including Lake Tana and Blue Nile wetland populations (Geremew and Triest, 2018), all assuming a stepping stone model between lakes. The strongest differentiation of *C. laevigatus* was found between any pair with that of Lake Magadi. There is a 100 km stretch between Lake Magadi and the nearest large water body (Lake Naivasha encompassing Lakes Oloiden and Sonachi), that is devoid of any perennial surface

Table 2
Pairwise comparisons of population genetic differentiation (F_{ST}) of *C. laevigatus*. All pairwise F_{ST} values were significant at $p < 0.001$ except when indicated with ^{NS}.

	Sadhana	Bogoria	Solai	Nakuru	Elem. Spring	Elementaita	Oloidien	Sonachi	Magadi
Sadhana	–								
Bogoria	0.324	–							
Solai	0.281	0.344	–						
Nakuru	0.378	0.492	0.163	–					
Elem. Spring	0.430	0.567	0.275	0.104^{NS}	–				
Elementaita	0.525	0.643	0.410	0.278	0.254	–			
Oloidien	0.389	0.577	0.382	0.227	0.221	0.310	–		
Sonachi	0.363	0.558	0.414	0.265	0.246	0.374	0.027 ^{NS}	–	
Magadi	0.765	0.623	0.775	0.811	0.874	0.895	0.806	0.775	–

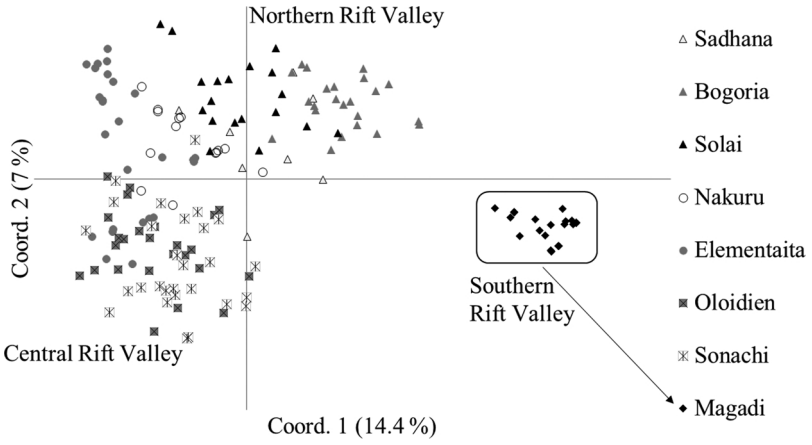

Fig. 1. PCoA at individual level for *C. laevigatus* showing a North to South gradient with little overlap between Northern to Central Rift Valley populations and fully separating Lake Magadi (Southern Rift Valley).

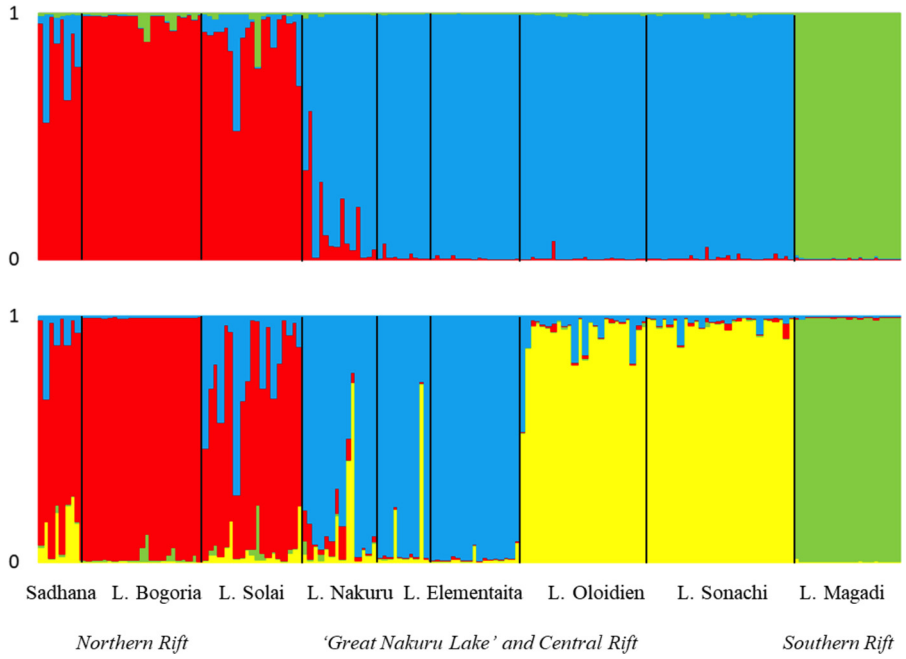

Fig. 2. Results of Bayesian analysis (STRUCTURE) for ΔK = 3 (above) and ΔK = 4 (below), clustering *C. laevigatus* individuals according to a North-South gradient of Rift Valley lakes. Vertical black lines delineate populations.

Table 3
Hierarchical AMOVA results for 4 regions (Df: degrees of freedom; SS: sum of squares and estimated variances) and F-Statistics between hierarchical levels with significance level *** $p < 0.001$.

Hierarchical level	Df	SS	Est. Var.	% variation	F-Statistic	Value
Among Regions	3	223.725	0.734	14%	F_{RT}	0.140***
Among Populations	5	74.859	0.298	6%	F_{SR}	0.066***
Among Individuals	154	773.492	0.800	15%	F_{ST}	0.196***
Within Individuals	163	558.000	3.423	65%	F_{IS}	0.189***
Total	325	1630.077	5.255	100%	F_{IT}	0.349***

water that would keep Lake Magadi connected to central Rift Valley lakes. It is possible that stronger historical connectivity exists between *C. laevigatus* from Lake Magadi with neighbouring southern systems such as Lake Natron, although this has not been tested.

The Eastern part of the Great Rift Valley widens (about 200 km wide) in the northern part of Kenya towards Lake Turkana due to a succession of splay faults and downwarps. A similar valley widening occurs on the eastern side of Lake Bogoria, which coupled with erosion in the area, could be an explanation for clustering *C. laevigatus* populations of the Sadhana Forest site (though outside the proper Rift Valley) with Lake Bogoria (King et al., 1972; Morley et al., 1992). Lake Solai clusters with the northern Rift Valley region although many individuals appear admixed with central Rift sites. Lake Solai is an important stop over for waterfowl migrating from Lake Bogoria to Lake Nakuru (De Bock et al., 2009). Dispersal agents of wetland sedges between current endorheic lakes could range from larger wildlife

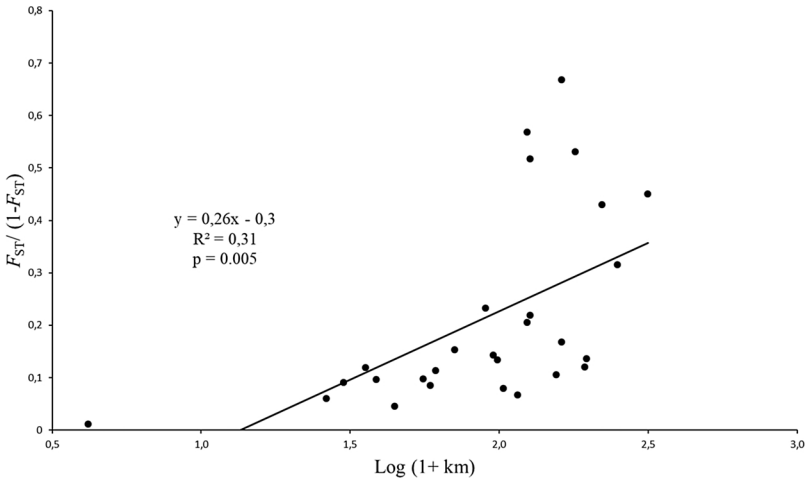

Fig. 3. Isolation-by-distance of *C. laevigatus* populations from alkaline-saline lakes in Eastern Rift Valley of Kenya, supporting a stepping stone model.

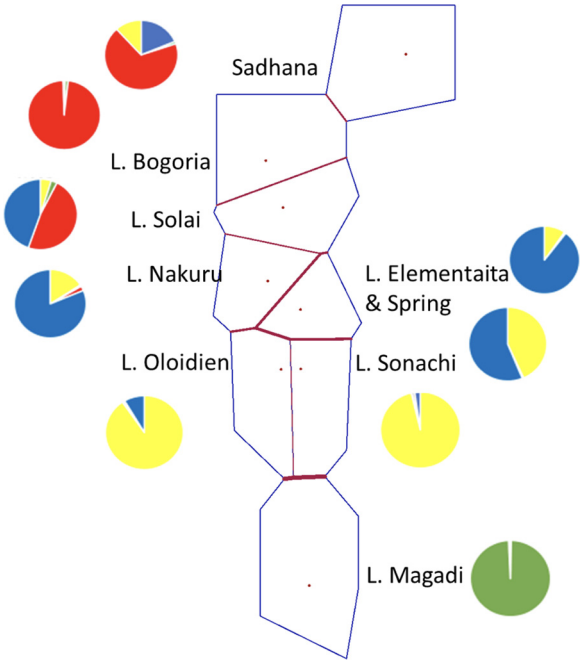

Fig. 4. Barriers in Rift Valley populations of *C. laevigatus* on basis of thirteen single loci F_{ST} values. Thickest boundary lines indicate strong differentiation. Pie chart colours refer to ΔK = 4 gene pools as shown in Fig. 2.

mammals (Onkware, 2000; Mwalyosi, 1983) to migratory waterfowl (Soons et al., 2008), but like other aquatic plants, especially hydrological connectivity could play a major role in shaping gene pools (Geremew and Triest, 2018; Geremew et al., 2018a; Terer et al., 2015; Triest et al., 2018).

4.4. Holocene hydrological connectivity of Great Nakuru Lake

The gene pool of the central Rift Valley region was made up of *C. laevigatus* populations from Lakes Nakuru, Elementaita, Oloidien and Sonachi, which could be explained by their historical inter-basin connectivity. The basins of Lakes Nakuru-Elementaita-Naivasha once were joined into one large paleolake, the 'Great Nakuru Lake', about 6000–12000 years B.P. This historical lake had an extensive area of about 800 km^2, a depth of 200 m and probably also connected to Lake Bogoria through an outlet near the Menengai crater (Bergner et al., 2009). A significant flow of water from Lake Naivasha towards the Elementaita-Nakuru basin was proposed on the basis of hydrological budgets during the Holocene, including subsurface water exchange (Dühnforth et al., 2006). This 'Great Nakuru Lake' period could explain *C. laevigatus* clustering into one gene pool since their populations were not separated for long. When considering $\Delta K = 4$, the central Rift Valley region was further divided into two gene pools, where Lakes Oloidien and Sonachi formed a cluster of their own, most likely due to the close proximity (3 km) between these two crater lakes (Harper et al., 1990). Paleolimnological studies showed that Lake Oloidien and Lake Naivasha used to be one lake but later separated and since then, the salinity of Lake Oloidien has been increasing (Verschuren et al., 2000).

Cyperus laevigatus of Lake Magadi in the southern Rift Valley, revealed more private alleles when compared to the other populations. This could be attributed to the mixing of two previously isolated populations during the beginning of the last interglacial and to the Pleistocene-Holocene period when the Magadi-Natron basin was part of the greater Oloronga paleolake (Hillaire-Marcel, 1987). *Cyperus laevigatus* populations have been reported on the shores of Lake Natron in Tanzania (Tibbett, 2015). This hypothesis would require a study of the populations found at the present-day Lakes Natron and Magadi for verification.

In conclusion, high resolution data from microsatellite loci demonstrated that *C. laevigatus* populations from the Rift Valley alkaline-saline lakes in Kenya showed high levels of clonal and genetic diversity, which was attributed to sexual reproduction modes, followed by very local persistence through vegetative growth. Few repeated clones were revealed and then, only within shortest distances along transects. Populations situated in sheltered locations showed fine-scale spatial genetic structure reflecting contemporary bulk seedling recruitment within a neighbourhood. A strong North–South genetic differentiation with both IBD and barriers was revealed along the Rift Valley region, thereby supporting a stepping-stone model of historical dispersal. Population division into several gene pools could be partly explained from historical inter-basin connections of the Great Nakuru Lake. However, each *C. laevigatus* population showed a high number of private alleles and allele diversity which could have implications for conservation as they each represent a potential hotspot of genetic diversity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2019.03.001.

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