MOLECULAR ECOLOGY

Supplemental Information for:

The population genomics of multiple tsetse fly (*Glossina fuscipes fuscipes*) admixture zones in Uganda

Norah P. Saarman, Robert Opiro, Chaz Hyseni, Richard Echodu, Elizabeth A. Opiyo, Kirstin Dion,
Thomas Johnson, Serap Aksoy and Adalgisa Caccone

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Supplementary Methods

ddRADseq library preparation methods from Gloria-Soria (2013)

500 ng DNA from each individual was digested with restriction enzymes NlaIII and MluCI with CutSmart Buffer (New England Biolabs, Carson City, USA) in a total volume of 50 µl. Each individual was labeled with Illumina adaptors carrying a unique barcode at the 5' end of the digested fragments, and were amplified by eight cycles of polymerase chain reaction (PCR). We then pooled a standardized quantity (80-100 ng) from 32 individuals and size-selected for 215 bp under the "tight" setting of a Blue Pippin electrophoresis platform (Sage Science) to create eight sequencing libraries.

ddRAD sequence processing and SNP calling

We built the original STACKS catalog with two of the highest quality representative reference-aligned samples from each population (n = 36), using the ref_map pipeline with default settings. SNPs in the remaining samples were then called against the existing catalog with the pstacks and sstacks scripts in genome mode (STACKS flag –g). We filtered SNP calls using the STACKS populations script to retain the following, biallelic SNPs, minimum depth of 5 reads (STACKS flag –m 5), SNPs genotyped in more than 70% of individuals per sample (STACKS flags –p 18 –r 0.7), and only the first SNP per ddRAD fragment (STACKS flag --write_single_snp), with PLINK file format output (STACKS flag --plink). We then used PLINK v1.9 (Purcell *et al,* 2007) to exclude individuals with more than 25% missing data (PLINK flag --mind 0.25), SNPs with less than 80% genotyping rate (PLINK flag --geno 0.02), and SNPs with less than a minor allele frequency of 0.2% (PLINK flag --maf 0.002) to retain only polymorphic SNPs present in more than one individual. We then created a VCF file by running STACKS again, this time using the final list of 251 individuals and the 55,267 ddRAD fragments that contained the 55,267 SNPs that passed all quality filters (STACKS flag --whitelist) and a minimum read depth of 5 (STACKS flag -m 5) with multiple output files for downstream analysis (STACKS flags --plink --vcf --fastphase --treemix --phylip), and again only included the first SNP per ddRAD fragment (STACKS flag --write_single_snp) for the downstream analysis. We also repeated the STACKs population script without this flag for estimates of per-sample diversity statistics that included all 158,537 SNPs (~2.85 SNPs per ddRAD fragment) and their surrounding invariable sites.

Microsatellite genotyping methods from Opiro et al. (2017)

Microsatellite loci GmmA06, GmmB20, GmmD15, GmmL03, GmmL11, A03b, B05, D05, D101, Gmm8, GpCAG29b, GpB20b, GpCAG133, GpC10b, Pg17, and Pg28 were amplified using the same primer combinations as Opiro et al. (2017; available at https://doi.org/10.1371/journal.pntd.0005485.s004). Amplifications were performed with fluorescently labeled forward primers (6-FAM, HEX and NED) using a touchdown PCR (10 cycles of annealing at progressively lower temperatures from 60˚C to 51˚C, followed by 35 cycles at 50˚C) in 13.0µl reaction volumes containing 2.6 µl of 5X PCR buffer, 1.1 µl of 10 mM dNTPs, 1.1 µl of 25mM $MgCl_2$ and 0.1 µl of 5 units/µl GoTaq (Promega, USA), 0.1 µl of 100X BSA (New England Biolabs, USA), 0.5 µl of 10 µM fluorescently-labeled M13 primer, 0.5 of µl 10 µM reverse primer, and 0.3 µl of 2 µM M13-tailed forward primer. For loci C7b and GmL11, 0.5 units of Taq Gold polymerase (Life Technologies, USA) were used instead of Promega GoTaq. PCR products were multiplexed in groups of two or three and genotyped on an ABI 3730xL Automated Sequencer (Life Technologies, USA) at the DNA Analysis Facility on Science Hill at Yale University (http://dna-analysis.yale.edu/). Alleles were scored using the program GENEMARKER v2.4.0 (Soft Genetics, State College, PA, USA) with manual editing of the automatically scored peaks.

mtDNA sequencing methods from Opiro et al. (2017)

We amplified a 490 bp fragment of the mitochondrial genome using primers COIF1 (5' – CCT CAA CAC TTT TTA GGT TTA G – 3') and COIIR1 (5' – GGT TCT CTA ATT TCA TCA AGT A – 3'), with an initial denaturation step at 95°C for 5 min, followed by 40 cycles of annealing at 50°C, and a final extension step at 72°C for 20 min. We used a reaction volume of 13.0 µl containing 1 µl of template genomic DNA, 2.6 µl of 5X PCR buffer, 1.1 µl of 10 mM dNTPs, 0.5 µl of 10mM primers, 1.1 µl of 25 mM MgCl2, and 0.1 µl (U/µL) of GoTaq polymerase (Promega, USA). The PCR products were purified using ExoSAP-IT (Affymetrix Inc., USA) as per the manufacturer's protocol. Sequencing was carried out for both forward and reverse strands on the ABI 3730xL automated sequencer at the DNA Analysis Facility on Science Hill at Yale University (http://dna-analysis.research.yale.edu/). Sequence chromatograms were inspected by eye and sequences trimmed to remove poor quality data using GENEIOUS v6.1.8 (Biomatters, New Zealand). The forward and reverse strands were used to create a consensus sequence for each sample, and the sequences trimmed to a length of 490 bp.

Analysis of molecular variance (AMOVA) among watersheds

We confirm that watersheds were a biologically relevant way to summarize the patterns of diversity found in *G. f. fuscipes* in Uganda with an analysis of molecular variance (AMOVA). We used the POPPR R library for the ddRAD SNP data set and ARLEQUIN v3.5 (Excoffer *et al*, 2010) for the microsatellite and mtDNA data sets. Computational limitations with the ddRAD SNPs AMOVA forced us to consider only ~2,000 SNPs, so we arbitrarily choose the 2,046 ddRAD SNPs with less than 0.05% missing data and assessed significance based on p-values calculated from 10,000 randomizations. We interpret the significant partitioning of molecular variance among the seven watersheds (Table S5) as evidence that genetic differentiation is explained by watershed beyond isolation by distance.

Supplemental details of the DAPC and FINESTRUCTURE analysis

For the DAPC analysis in "adegenet" R package, the "find.cluster" function was used with all principal components (PCs) available, followed by the main "dapc" function with just four PCs, and the most informative *K* was assessed with the Bayesian Information Criterion (BIC).

For the FINESTRUCTURE v. 2.0.7 (Lawson *et al,* 2012) analysis all 55,267 SNPs were phased and imputed on a scaffold-by-scaffold basis using FASTPHASE v. 1.2 (Scheet and Stephens 2006). The linked model was used, with the c-factor estimated in the first step, and with the following parameters, 750,000 iterations of the MCMC with 375,000 burn-in steps and 1000 samples retained, 20,000 tree comparisons and 500,000 steps of tree maximization. The MCMC trace files were viewed to confirm the stability of all parameters, and the results were visualized in R with the scrips provided by FINESTRUCTURE.

Supplemental details of the TreeMix analysis

We assessed phylogenetic relationships with the program TreeMix. Instead of filtering SNPs to account for LD before the analysis as we did in the other population structure analyses, we used all 55,267 SNPs and accounted for LD in sliding window stretches of 100 SNPs at a time (TreeMix flag -k 100). The window size of 100 SNPs equates to approximately 100 kb genome blocks (estimated based on Pi_A and the total 374.8 giga base pairs length of the *G. f. fuscipes* genome), which is a value that far exceeds the known extent of LD in *G. f. fuscipes* (Gloria-Soria *et al,* 2013; 2017). Confidence in the final TreeMix ML tree was judged with 1,000 bootstrap replicates using a python wrapper (https, // github.com / mgharvey / misc_python / blob / master / bin / TreeMix / treemix_tree_with_bootstraps.py) for TreeMix and the "sumtrees" function of DendroPy v. 4.3.0 (Sukumaran and Holder, 2010), with modifications that allowed for migration events (Supplemental Python Code).

Supplemental details of the MIGRAINE analysis

MIGRAINE analysis modeled demographic history assuming an exponential change in population size from the ancestral time point continuing until the current time point, which is the basic coalescence model (Leblois *et al*, 2014). This analysis used the microsatellite data set because the one based on ddRAD SNPs was too computationally intensive to complete. We assumed a generalized stepwise mutation, a realistic yet simple mutation model that has been shown to reduce false positives (Peery *et al*, 2012; Leblois *et al*, 2014), and assumed the mutation rate (μ) of 1.0E-4 per generation per locus, which is a realistic mutation rate for microsatellites in this taxonomic group (Ciosi *et al,* 2014; Chapuis *et al*, 2015). The scaled parameter representing genetic diversity was estimated for the current sample (θ) and the hypothetical ancestral sample (θ_{anc}), and population size changes were tested for using the "OnePopVarSize" model (Leblois *et al*, 2014), using five iterations of 500 points and 1,000 runs per point. Significant population size changes were determined based on non-overlapping 95% confidence intervals of θ and θ_{anc}. We converted scaled parameters (θ and θ_{anc}) into biological ones with the equations $\theta=2N\mu$ and $\theta_{anc}=2N_{anc}\mu$, respectively.

Supplemental details of the ABC analyses

Preliminary analysis – The preliminary ABC analysis aimed at establishing realistic time priors. To do this we only used the microsatellite data set because models based on SNP datasets are difficult to use for preliminary analysis for several reason. They are a random draw of loci with all the possible evolutionary histories (including a random sample of loci with introgressed alleles, ancestral polymorphisms, slow and fast mutation rates, synonymous and non-synonymous mutations, and from across the full site-frequency spectrum). Subsequently, when posteriors from SNP-based analysis are unrealistic, the combination of having an unknown mutation rate and model, and the interdependence of posterior estimates, makes it impossible to know which prior to alter in the next analysis. Microsatellites, on the other hand, have well known minor allele frequencies, as they were developed for their high minor allele frequencies and high diversity (Brown *et al,* 2008), and have well-described mutation models (Ciosi et al., 2014; Chapuis *et al*, 2015). All priors were based on best practices (Cournuet *et al* 2014) and following previous studies wherever possible (Ciosi et al., 2014). The first preliminary analysis with unrestricted time priors created unrealistic posterior time estimates that were truncated by zero (Figure S13). Thus, we completed two more preliminary ABC analyses with increasing specificity (Table S3) that matched the biogeographic history of *G. f. fuscipes* (see Figure 3) until time priors were no longer severely truncated by zero.

Microsatellite priors and summary statistics – For the microsatellites (for both preliminary and the final analyses) we used the default Generalized Stepwise Mutation Model (Estoup et al., 2002) with a mutation rate of 1E-3 to 1E-4, and a starting parameter of the geometric distribution (*P*) of 0.22 (Table S3). We chose these default priors since the simplest models tend to perform sufficiently (Cournuet et al., 2006), and a similar model was shown to be realistic in ABC analysis in closely related *Glossina pallidipes* (Ciosi et al., 2014). For this dataset as summary statistics we used mean gene diversity (Nei, 1987), number of alleles, and M index (Garza and Williamson, 2001; Excoffier et al., 2005) across all loci for all samples, and mean pairwise F_{ST} distances (Weir and Cockerham, 1984) for each pair of samples.

ddRAD priors and summary statistics (unsuccessful analysis) – We also attempted to run the same analysis with the ddRAD dataset, but were unsuccessful in parameterizing the mutation models (Figure S14). We first trimmed the SNP dataset to excluded highly linked SNPs by starting with the 33,057 LD-filtered SNPs used for clustering analysis. Then we excluded outlier loci with the R library PCAdapt (Luu et al. 2016), as these loci could be behaving in a non-neutral fashion. This reduced the starting ddRAD data set included in the ABC analysis to 27,971 SNPs. For the ddRAD SNPs, the only method available was a simplified and efficient algorithm that does not require or accept any parameterization of the mutation model (Cornuet et al. 2014) beyond a minor allele frequency (MAF), which we restricted as little as possible to the MAF that equates to 0.05 (DIYABC flag <MAF=hudson>). For this dataset as summary statistics we used mean gene diversity (Nei, 1987) across polymorphic loci and across all loci for each sample, as well as the mean pairwise F_{ST} distances (Weir and Cockerham, 1984) for each pair of samples.

Supplemental details of final ABC scenarios, and method of choosing the best scenario

Details of Scenario 1 – Scenario 1 considers the hypothesis that the major genetic breaks among the three genetic clusters were created by major river reorganizations at ~400 ka, ~20-40 ka, and at ~10-20 ka (Figure 2), and that the signal of admixture in zone "b" in the Okole River watershed was created by recent secondary contact between lineages that diverged during the last glacial maximum ~10-20 ka (Figure 2c). Under Scenario 1 (Figure 3a), divergence between the northwest/northeast and west/south genetic clusters (Figure 1) occurred during the first major reorganization of river systems ~400 ka (Figure 2a). Divergence between the west and south (Figure 1) occurred during the second reorganization of river systems ~20-40 ka (Figure 2b). Divergence between the northwest and northeast (Figure 1) occurred during the final river reorganization and multiple desiccation periods at the end of the last glacial cycle ~10-20 ka (Figure 2c), followed by recent secondary contact and admixture between them in the Okole River watershed (zone "b"). Range expansion after divergence could have allowed for hybridization between these two genetic units, allowing much more recent admixture in the last

135 years (t1 in Figure 3) between these two populations to create the Okole River sample (at a rate varying from 0 to 1) current-day pattern of genetic admixture observed in the Okole River (Opiro *et al* 2017).

Details of Scenario 2 – Scenario 2 considers the hypothesis that, as in Scenario 1, the major genetic breaks between the northern, western, and southern clusters were created by major river reorganizations. However, contrary to Scenario 1, in Scenario 2 (Figure 3b), the signal of admixture in zone “b” in the Okole River watershed was caused by incomplete divergence, which could create a false signal of admixture in clustering analyses (Serre and Paabo, 2004; Rosenberg *et al*, 2005; Frantz *et al*, 2009; Schwartz and McKelvey, 2010). Under Scenario 2 (Figure 3b), the final river reorganization and multiple desiccation periods at the end of the last glacial cycle ~10-20 ka (Figure 2c) would not have caused a lasting signal of divergence between the northwest and northeast (Figure 1). Instead, under Scenario 2, migration-drift equilibrium was established and maintained a panmictic gene pool until very recently. To test this scenario in an ABC framework, we set priors that constrained the divergence of the northwest, zone “b”, and northeast to within the last 135 years. We used two different topologies to allow for the possibility that the Okole River (zone “b”) shared more gene flow with either the northwest Albert Nile (topology 2a in Figure 3b) or with the northeast Lake Kyoga (topology 2b in Figure 3).

Details of Scenario 3 – Scenario 3 considers the hypothesis that, as in Scenario 1 and 2, the major genetic breaks between the western and southern watersheds were created by major river reorganizations. However, contrary to Scenario 1 and 2, in Scenario 3 (Figure 3c) the signal of admixture in zone “b” in the Okole River watershed was created by a severe bottleneck event. This event could have reduced the genetic diversity in the northeast to a subset of that found in zone “b”, creating a false signal of admixture (Falush *et al*, 2016). Under Scenario 3 (Figure 3c), divergence between the northwest and northeast (Figure 1) would have occurred during the last glacial cycle ~10-20 ka (Figure 2c), followed by a severe population bottleneck in the northeast that created a false signal of admixture in the intermediate lineage (Falush *et al*, 2016), in this case zone “b”. To test this scenario in an ABC framework, we set priors that constrained the divergence of the northeast and northwest to the approximate time of the last river reorganization and desiccation events ~10-20 ka (Figure 2c, t2 in Figure 3), followed shortly by a strong population bottleneck in the northwest within the last 5 ka.

Goodness of fit and comparison of alternative scenarios – We ran principal components analysis with all summary statistics for each data type to visualize closeness of fit of the prior and posterior distributions to the observed dataset. We also computed the “posterior predictive error” using simulated pseudo-observed datasets (PODs) for which the true scenario ID and parameter values are known, and estimating the proportion of wrongly identified scenarios in the PODs from the simulated datasets closest to the observed dataset (Cournuet *et al.,* 2014). To compare scenarios and to choose the best scenario, we computed posterior probabilities of each scenario by the polychotomic weighted logistic regression method described by Fagundes et al. (2007) and Beaumont (2008), and computed 95% confidence intervals (Cornuet et al., 2008).

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Supplementary Figures

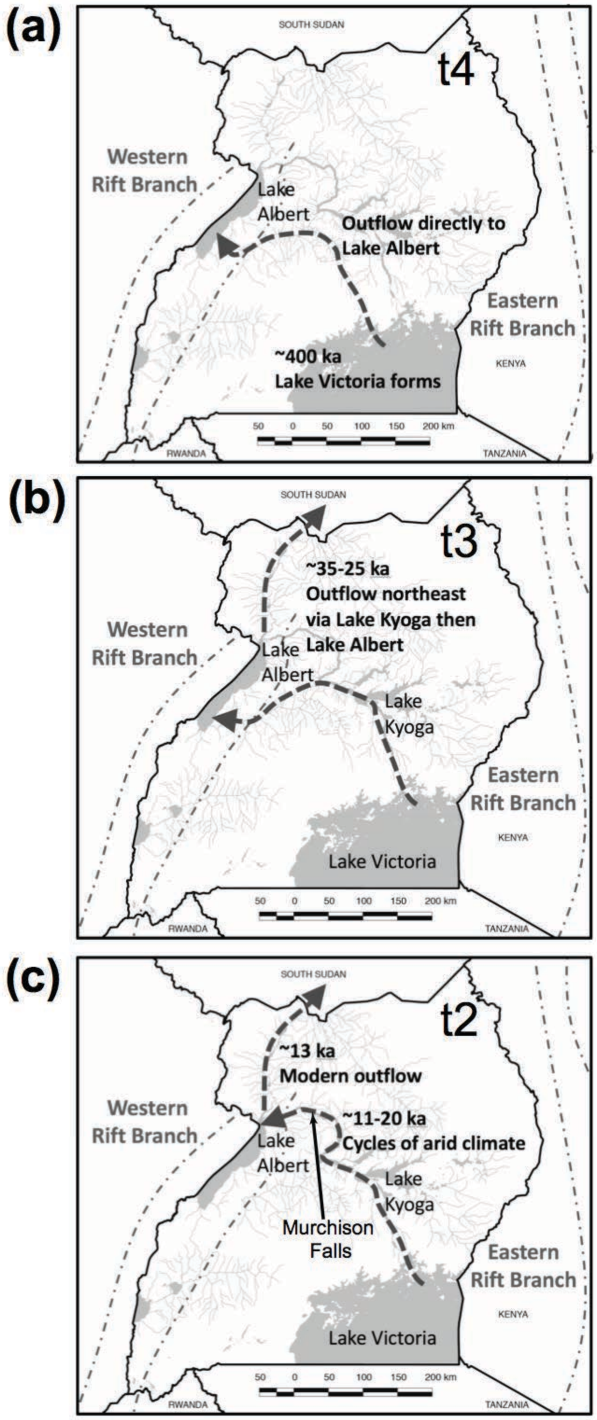


Figure S1. Schematic of the major reorganization of river systems in Uganda during the last ~400 thousand years (ka), as summarized by Danley et al (2012). The three panels are labeled t4, t3, and t2 to relate the approximate times of geologic events to the time priors used for the preliminary ABC analysis and the final ABC analysis comparing the three competing scenarios (Figure 2). Dashed grey lines indicate the current placement of the Western Rift Branch and the Eastern Rift Branch of the African Rift System, light grey areas indicate the present-day rivers and lakes, dashed arrows indicate the approximate route and direction of major water flow during each time frame. **(a)** Lake Victoria began to backfill and established a connection with the west branch of the Nile ~400-100 ka (Figure 2: t4), **(b)** the Lake Victoria altered course to flow through Lake Kyoga by ~35–25 ka (Figure 2: t3), and **(c)** the Lake Victoria altered course again to connect to the Nile through Murchison Falls by ~13 ka, and there were several cycles of arid climates that completely dried lake Kyoga and Lake Victoria (Figure 2: t2).

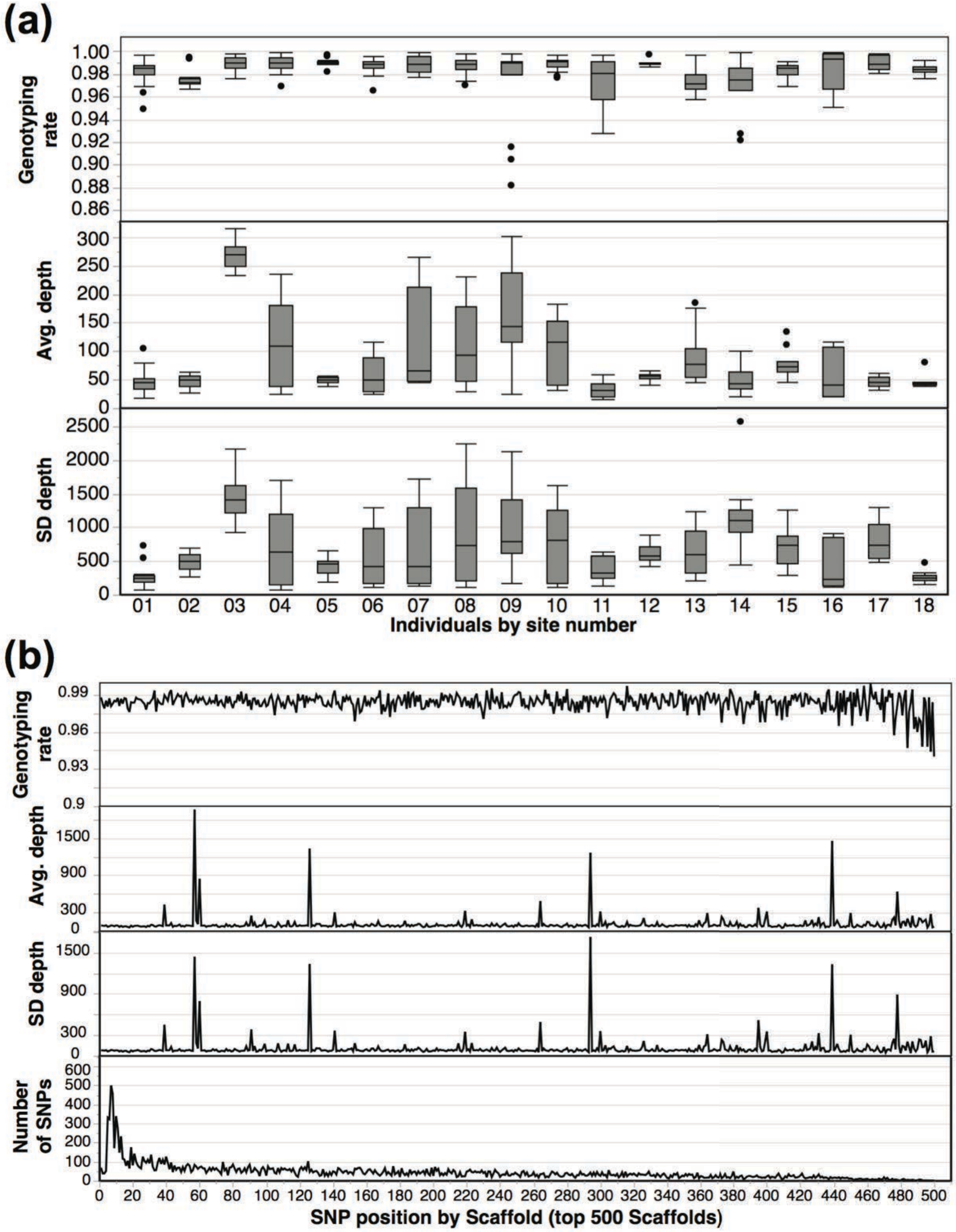


Figure S2. ddRAD SNP genotyping rate and read depth statistics. (A) Quantile box plots showing minimum, 25% quantile, mean, 75% quantile, and maximum of the genotyping rate, average depth, and standard deviation (SD) of depth among each site (labeled by site number in Figure 1) across all 55,267 ddRAD SNP loci included in the study. (B) Line plot displaying the same statistics (genotyping rate, average depth, and SD of depth), as well as the number of SNPs present on each scaffold, this time averaged among each locus across all individuals, with only the 21,377 SNPs that fall on the 500 Scaffolds containing the most SNPs (top 500 Scaffolds) displayed.

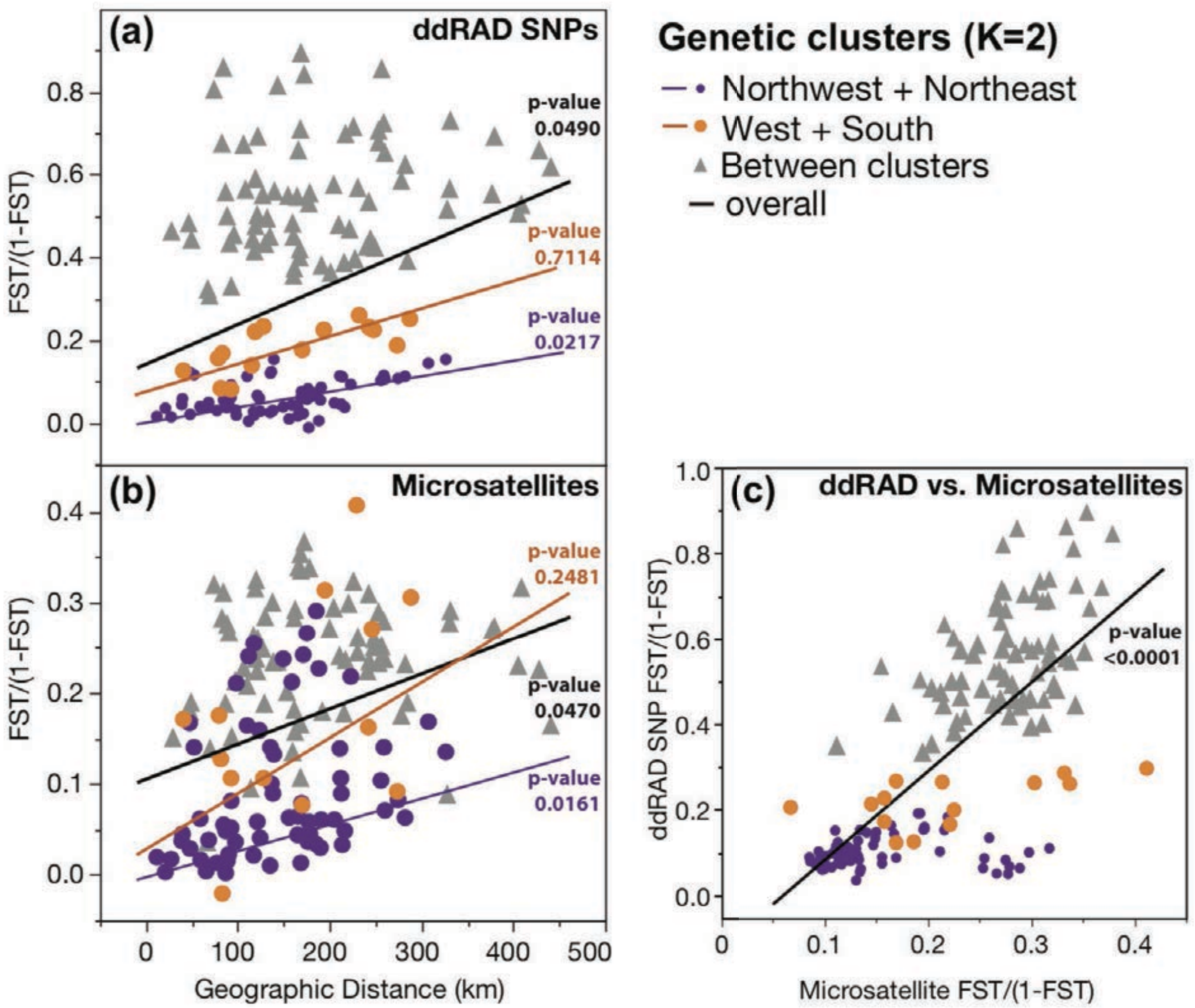


Figure S3. Pairwise differentiation (Wright 1951; Weir and Cockerham 1984) corrected for finite population size [$F_{ST}/(1- F_{ST})$] versus geographic distance (km), showing correlation of **(a)** differentiation based on 55,627 ddRAD SNPs with geographic distance overall (p-value 0.047) and among the two units that all clustering analysis agreed upon at K=2 (p-value 0.0217 for the northwest and northeast, p-value 0.7114 for the west and south), **(b)** differentiation based on 16 microsatellites with geographic distance overall (p-value 0.049) and among the two units at K=2 (p-value 0.0161 for the northwest and northeast, p-value 0.2481 for the west and south), and **(c)** the ddRAD SNP and microsatellite based differentiation with each other overall (p-value <0.001). For the ddRAD SNPs F_{ST} was estimated in the StAMPP R package (Pembleton et al., 2013), and for the microsatellites F_{ST} was estimated in FSTAT 2.9.3 (Goudet 1995). Geographic distances were generated using the Java based geographic matrix generator v1.2.3 (Erst, downloaded November 2017). Mantel tests for significance of each relationship and partition of the data were tested with 10,000 randomizations of the data using the adegenet v. 2.0.1 R library (Jombart et al., 2008, 2011).

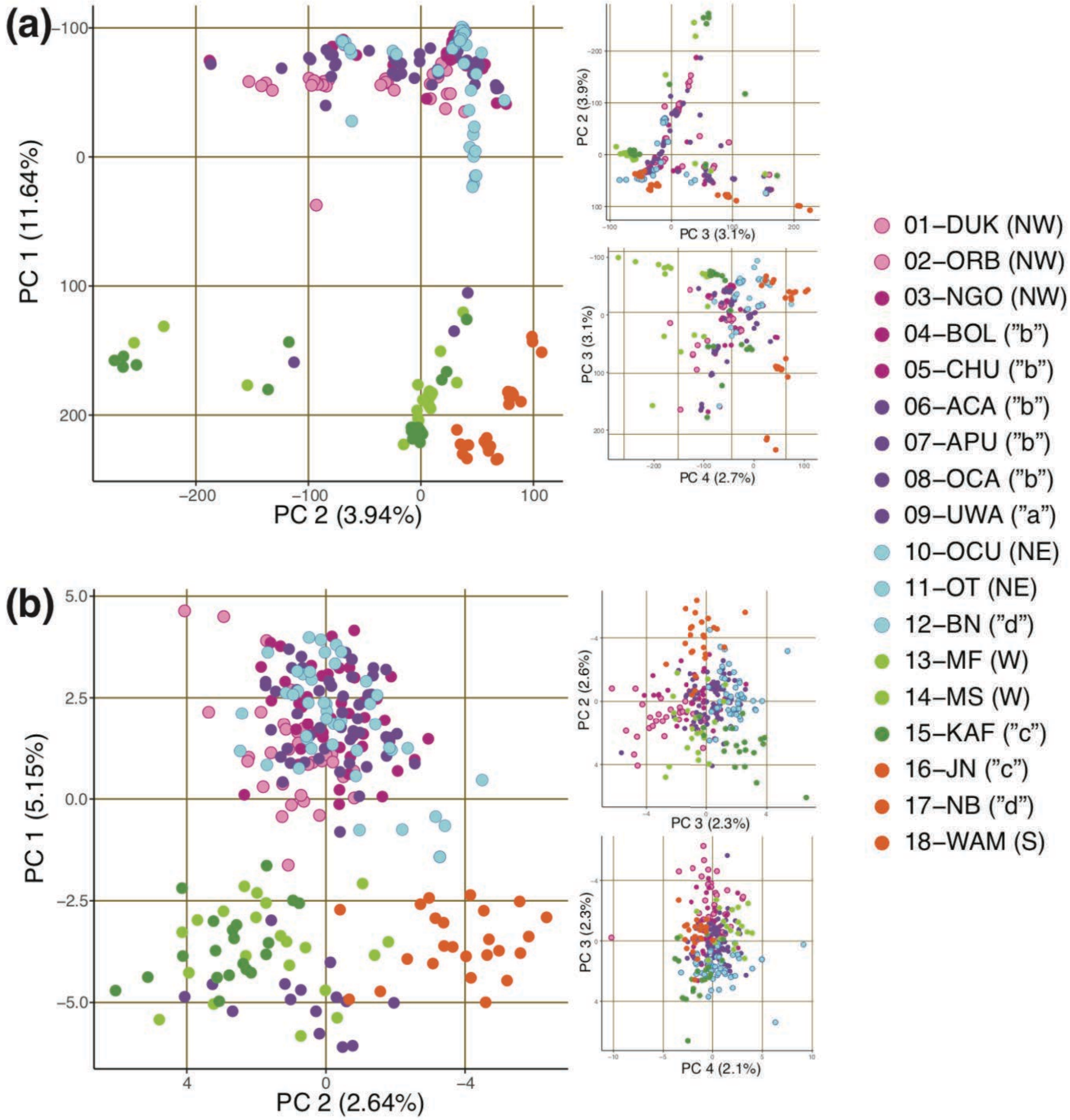


Figure S4. Principle components analysis (PCA) analysis plots for **(a)** 55,267 ddRAD SNPs, and **(b)** 16 microsatellites displaying the first four principal components. Each individual is indicated with a point colored by watershed of origin (Albert Nile: pink, Ashwa River: magenta, Okole River: purple, Lake Kyoga: blue, Lake Albert: light green, Kafu River: dark green, Lake Victoria: orange) with the site code (1-18) and the expected genetic unit in parentheses on the right. The percent variance explained by each principal component (PC) is indicated in parentheses on each axis.

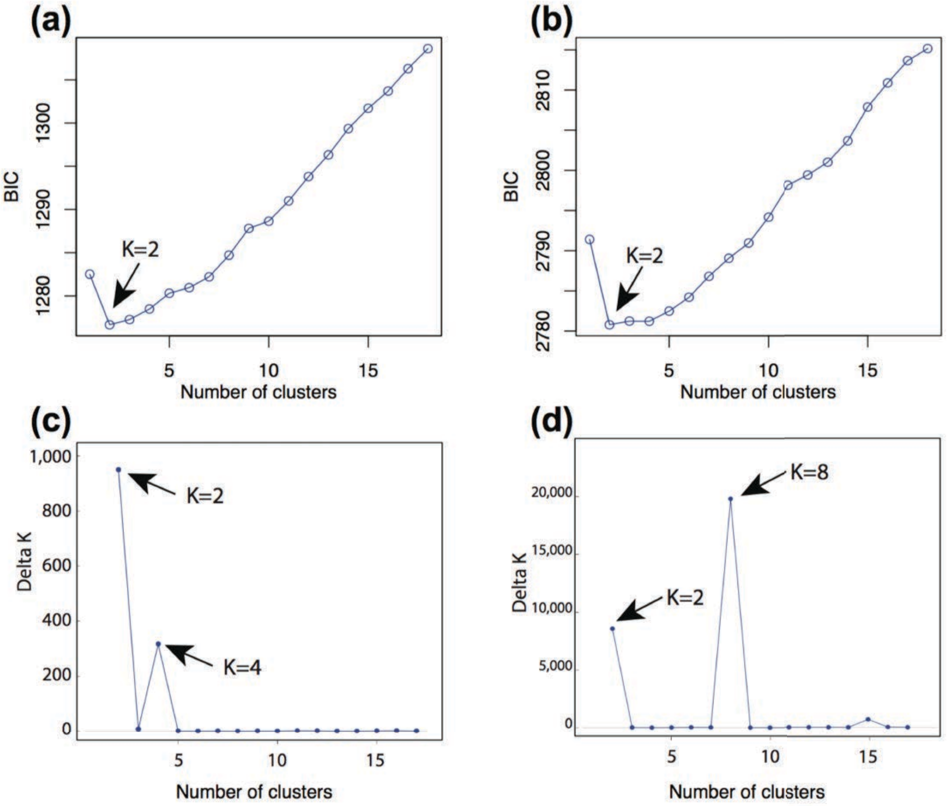
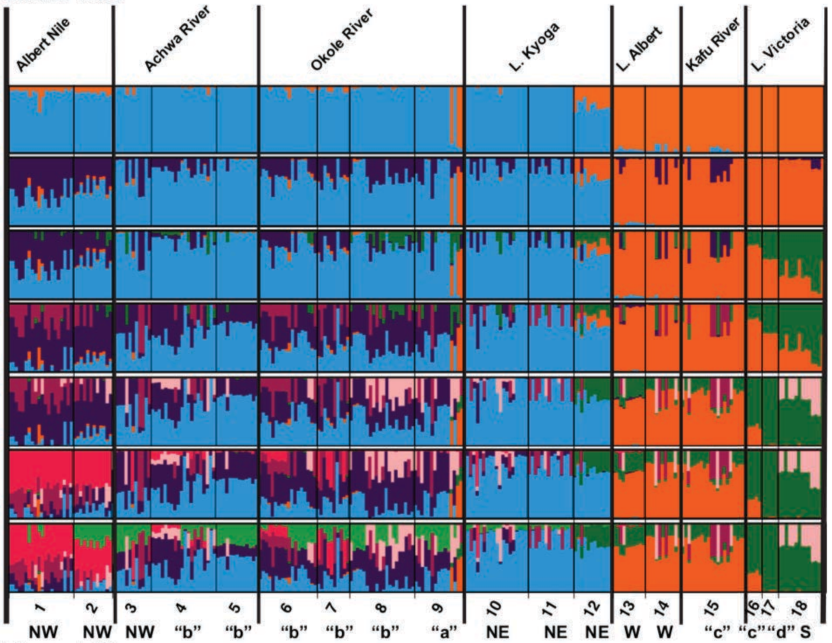


Figure S5. Clustering analysis assessments of the number of population clusters (*K*) 1-18 present in the ddRAD SNP and the microsatellite datasets. Optimal *K* for the **(a)** ddRAD SNPs and **(b)** the microsatellites from clustering analysis using Discriminant Analysis of Principal Components (DAPC; Jombart et al., 2010, 2012), for the **(c)** ddRAD SNPs and **(d)** the microsatellites from clustering analysis using STRUCTURE v2.3.4 (Pritchard et al. 2000). The arrows point to the K-values that explain the largest component of the structure present in the sample.

(a) ddRAD SNPs



(b) Microsatellites

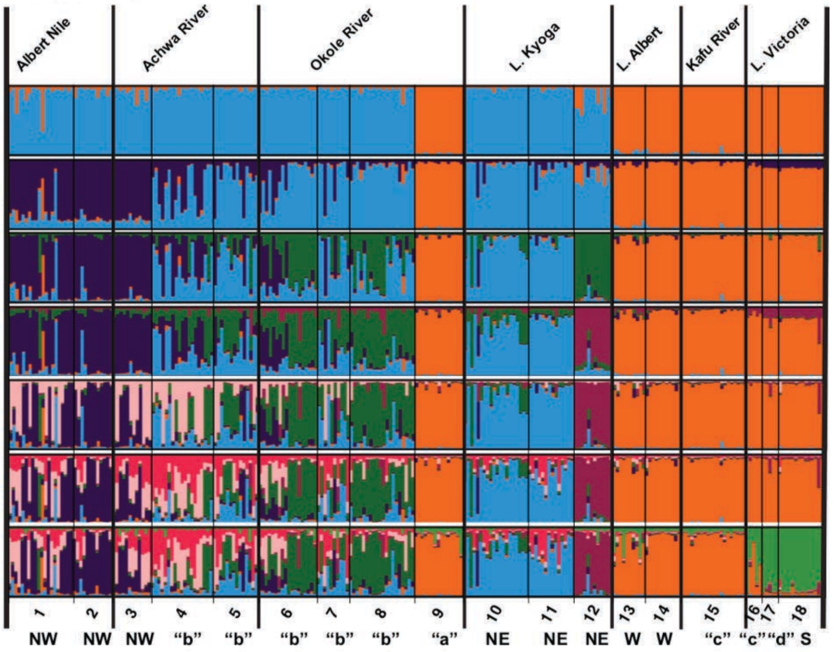


Figure S6. Plots showing the STRUCTURE v2.3.4 (Pritchard et al., 2000; Falush et al., 2003) results with *K*=2-8 based on **(a)** 33,627 unlinked ddRAD SNPs, and **(b)** 16 microsatellites. Each bar represents the composite genotype of one individual fly, and the colors within the bar reflect the probability of assignment (q-value) of that individual. Thick black lines identify the watersheds of origin. Watersheds are listed above, sampling sites (1-18) and the expected genetic units for each individual are listed below.

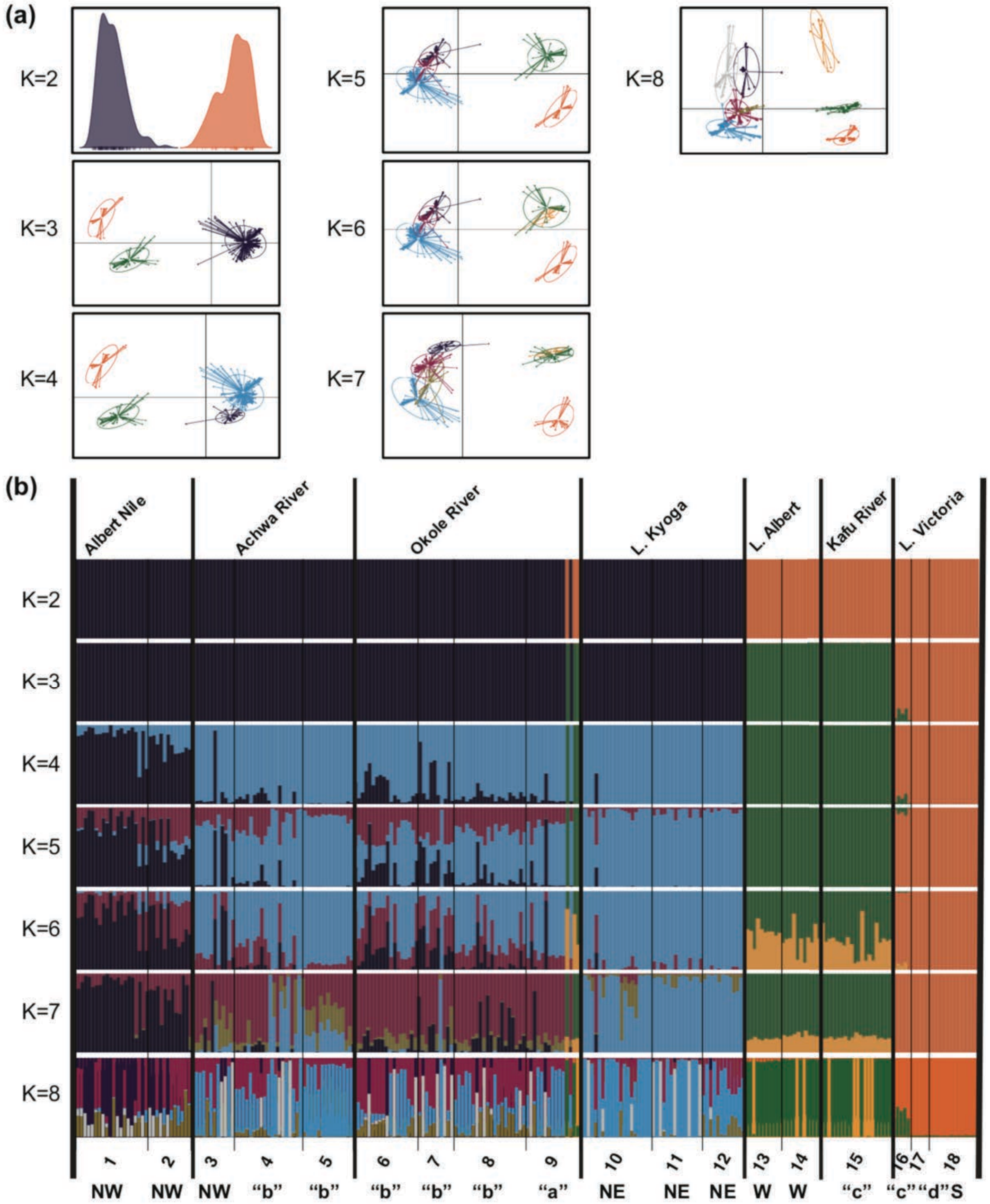


Figure S7. Results of the discriminant analysis of principal components (DAPC) from 33,627 unlinked ddRAD SNPs using the adegenet R library (Jombart et al., 2010, 2012). **(a)** Results of the “find.clusters” algorithm with *K*=2-8, where the ellipse encompasses 95% of the variance for each found cluster and each individual is connected by a line to the centroid. **(b)** Bar plot of each individual’s probability of assignment (q-values) to the *K* found clusters displayed in the top panel. Sampling sites (1-18) and the expected genetic unit for each individual are listed below.

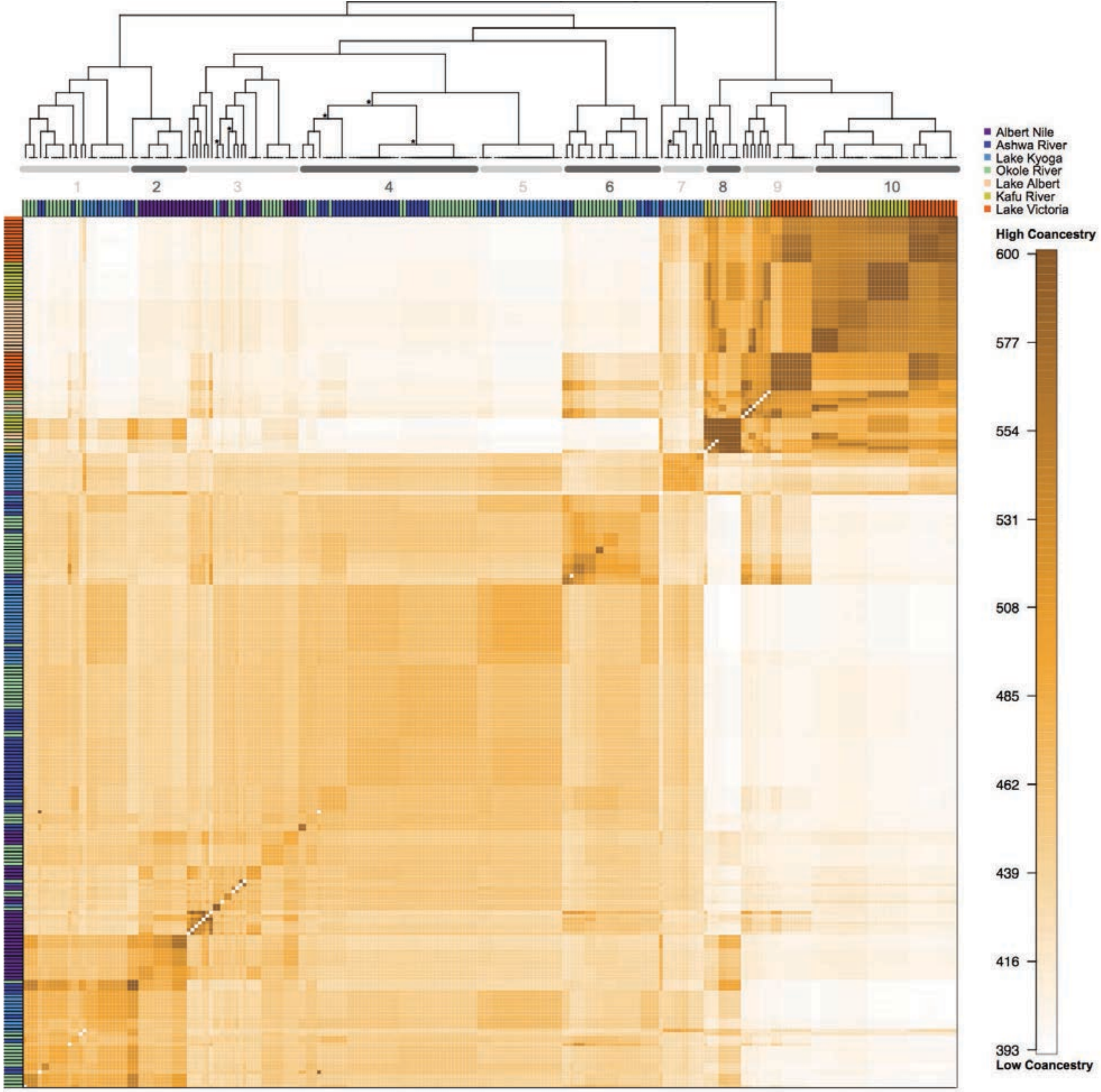


Figure S8. Co-ancestry heat map from all 55,267 ddRAD SNPs where white and brown, respectively, denote lower and higher co-ancestry identified by FINESTRUCTURE v. 2.0.7 (Lawson et al. 2012). A bifurcating tree and the 10 resulting populations (grey horizontal bars) are shown on the top and are titled 1-10. On the bifurcating tree, MCMC posterior assignment probabilities greater than 0.9 are marked *. Each individual is colored by watershed of origin along the top and left side of the heat map, and is generally ordered from North on the left to South on the right. Colors are based on watershed of origin: The Albert Nile (northwest) in pink, the Ashwa River in magenta, the Okole River in purple, Lake Kyoga (northeast) in blue, Lake Albert (west) in light green, Kafu River in dark green, Lake Victoria (south) in orange. See Table 1 for the genetic units and admixture zones for each watershed. See Table S8 for details of assignment of each individual. All 55,267 ddRAD SNPs were used without prior filtering for linkage-disequilibrium (LD) because the algorithm takes LD into account.

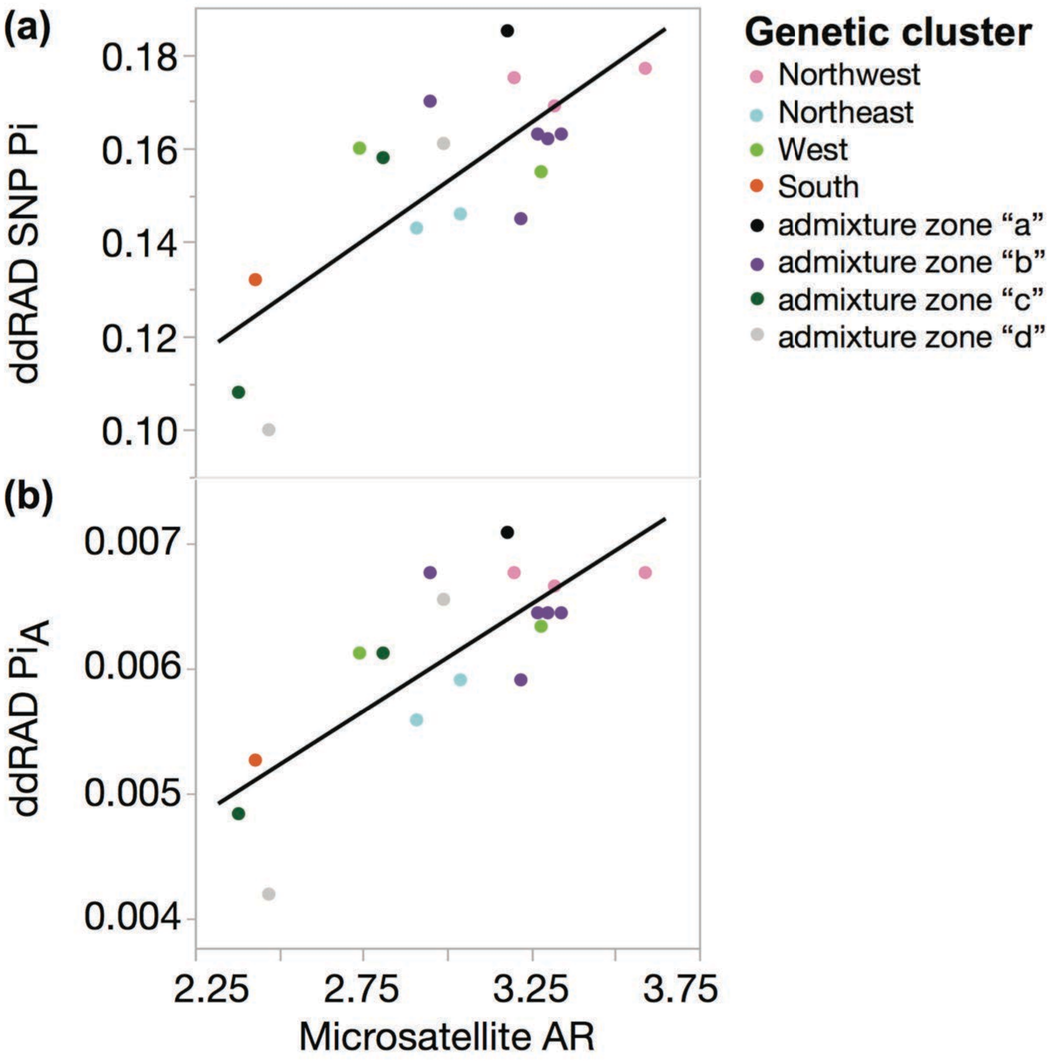


Figure S9. Genetic diversity of *Glossina fuscipes fuscipes* samples from Uganda. Plots showing correlation of microsatellite allelic richness (AR) with nucleotide diversity across 55,627 ddRAD loci considering **(a)** only polymorphic SNPs (Pi; p-value <0.001), and **(b)** all sites at both variable and invariable positions in each ddRAD fragment (Pi_A; p-value <0.001). Each point represents one of the 18 samples, colored by the two main genetic units identified in all clustering analysis (K=2) genetic unit/admixture zone. Nucleotide diversity was estimated using the populations script of the Stacks v. 1.34 software (Catchen et al. 2011). Allelic richness was estimated using PopGenReport v. 1.0.2 (Adamack and Gruber, 2014; Gruber and Adamack, 2015) and the linear regression was completed in the core stats R package.

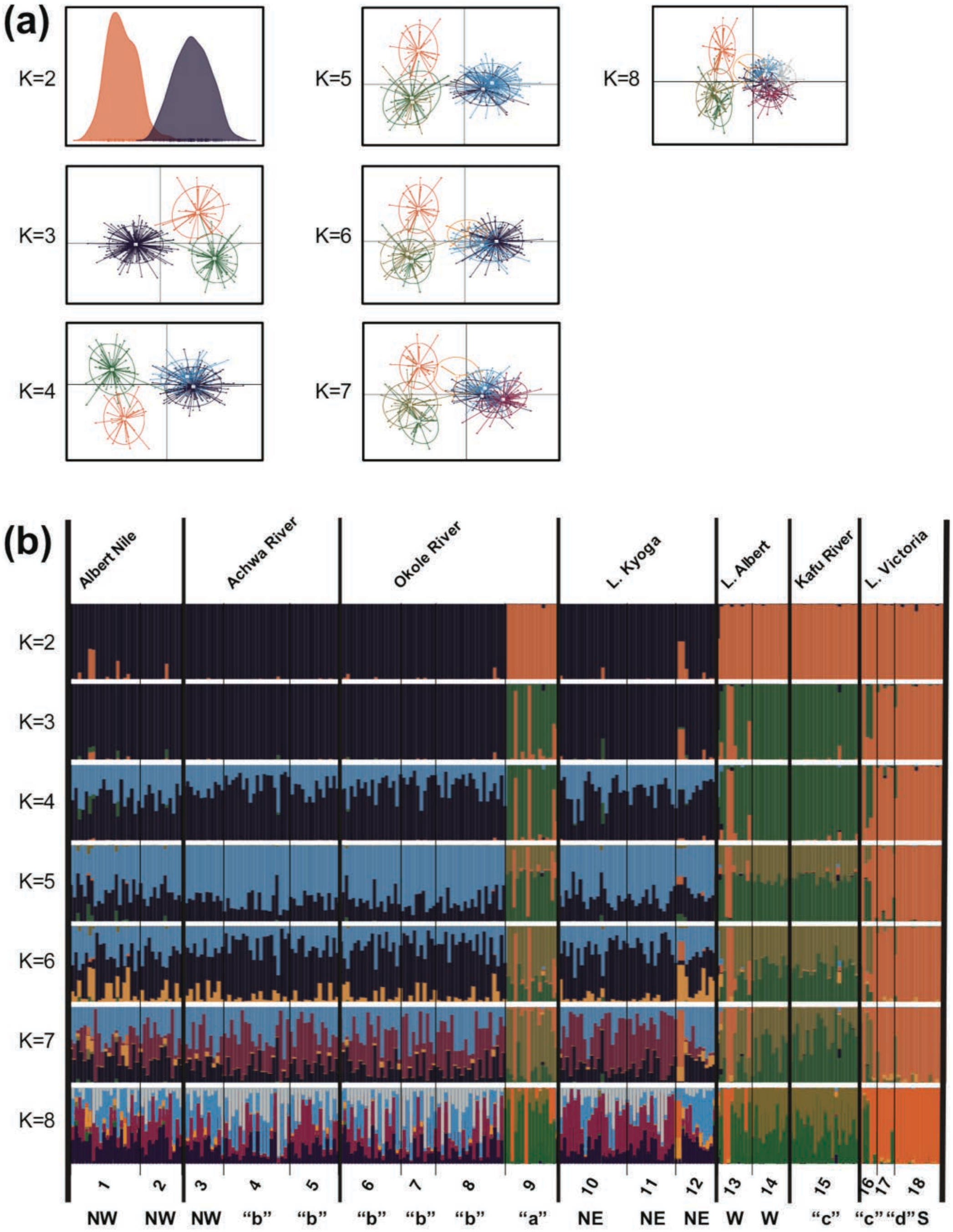


Figure S10. Microsatellite based results of the discriminant analysis of principal components (DAPC) using the adegenet R library (Jombart et al., 2010, 2012). **(a)** Results of the "find.clusters" algorithm with *K*=2-8. Different colors represent different found clusters, with dots showing individuals assigned to that cluster connected by a line to the centroid and ellipse encompass 95% of the variance within each cluster. **(b)** Bar plot of the probability of assignment (q-value) to *K* clusters, where each bar represents the composite genotype and assignment of one individual fly to each of *K* clusters displayed in panel A. Sampling sites (1-18) and the expected genetic unit for each individual are listed below.

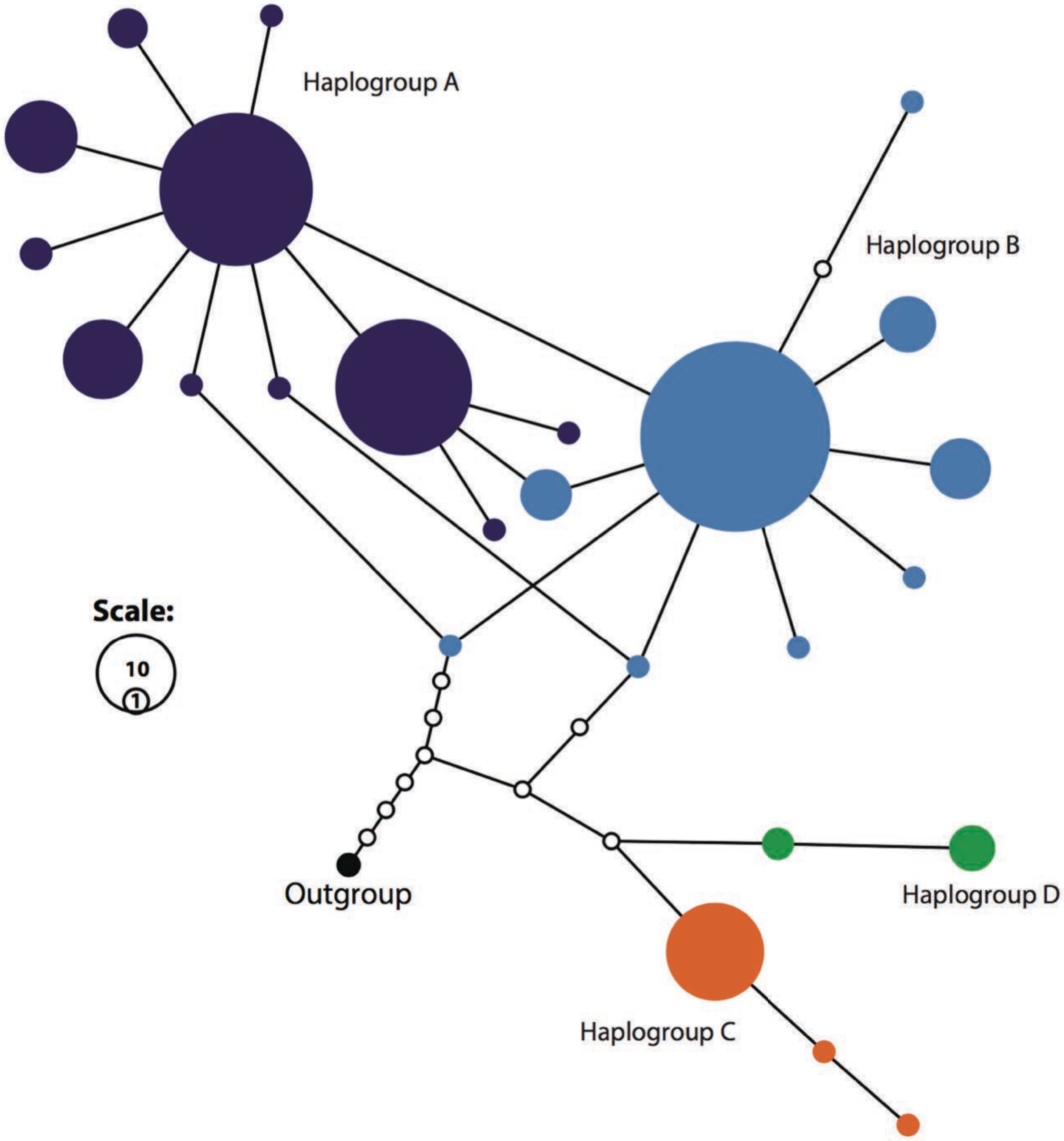


Figure S11. Haplotype network for a 490 bp mtDNA COI-COII gene fragment, where haplotypes are represented by circles that are sized proportionally to frequency and shaded to represent clades composed of equivalent mutational steps. Black dots represent unsampled haplotypes. Colors represent groups of related haplotypes (Haplogroup A in purple, Haplogroup B in blue, Haplogroup C in orange, and Haplogroup D in green).

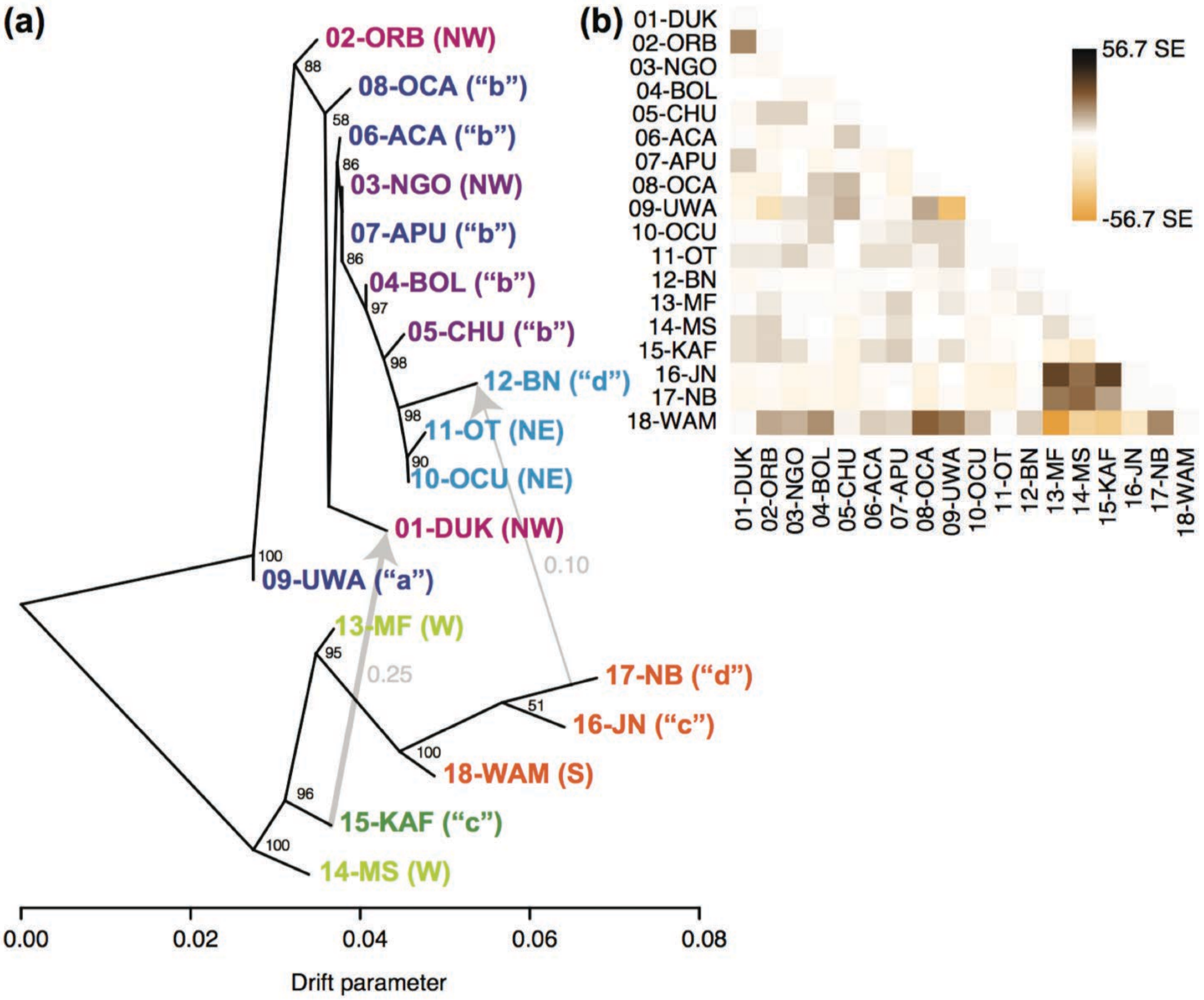
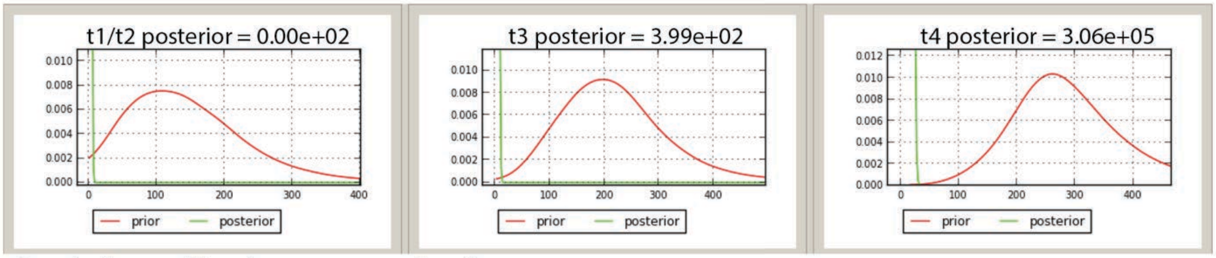
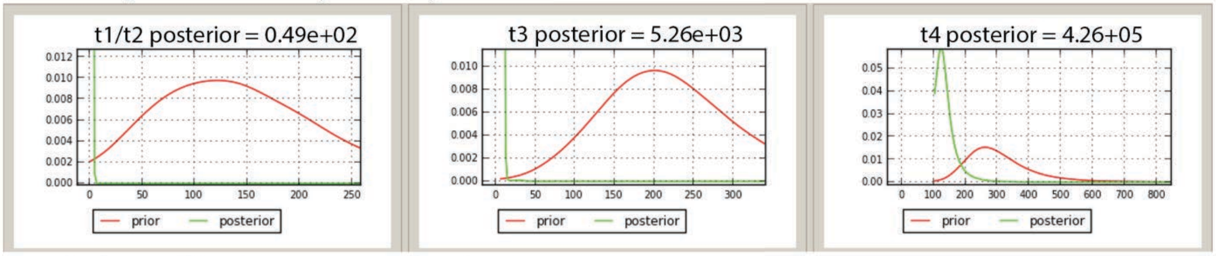


Figure S12. TreeMix (Pickrell and Pritchard, 2012) results based on 55,267 ddRAD SNPs. (**a)** Maximum-likelihood tree of population relationships with the 18 site codes and the geographic region or admixture zone of origin colored by watershed (Albert Nile: pink, Ashwa River: magenta, Okole River: purple, Lake Kyoga: blue, Lake Albert: light green, Kafu River: dark green, Lake Victoria: orange), with support values based on 1,000 bootstrap replications. Inferred migration events (directions and rates) are shown in gray (thickness of the lines represents relative migration strengths). The X axis provides the "drift parameter" (average standard error of the entries in the sample covariance matrix), which corresponds to the distance proportional to the amount of genetic drift that has occurred on the branch over 2Ne generations. (**b)** The residual covariance (scaled by the average standard error) across all population pairs colored as described in the palette on the right. Values above zero (in brown) are candidates for additional admixture events beyond what was captured by the migration estimates in panel a.

(a) Serial preliminary analysis 1



(b) Serial preliminary analysis 2



(c) Serial preliminary analysis 3

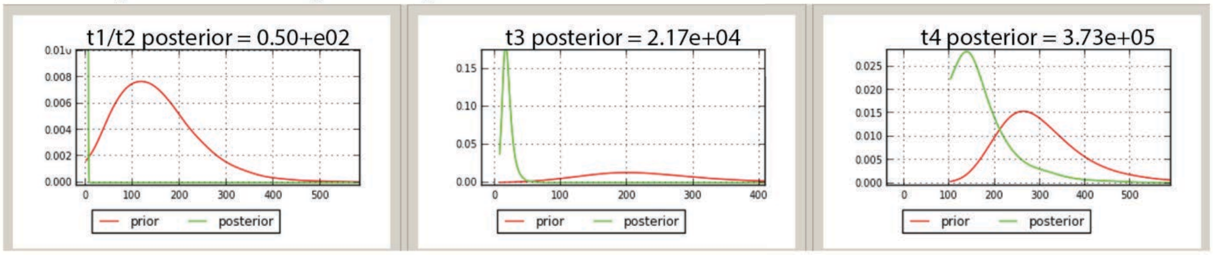


Figure S13. Results from microsatellite-based serial preliminary ABC analyses **(a)** 1, **(b)** 2, and **(c)** 3 wherein priors were specified with greater and greater specificity (Table S3) that matched the biogeographic history of *G. f. fuscipes* (see Figure 3) until time priors were no longer severely truncated by zero (in serial preliminary analysis 3). In the plots, the distribution of prior and posterior time estimates (t1/t2, t2, and t3) are shown in red and green, respectively, and were scaled by the average N_e and generation time (8 per year; Krafsur *et al.* 2009). Unscaled posterior median time estimates (in approximate years before sampling efforts) are reported along the top of each plot. All ABC analysis was performed in DIYABC v2.0.4 (Cornuet *et al.,* 2014).

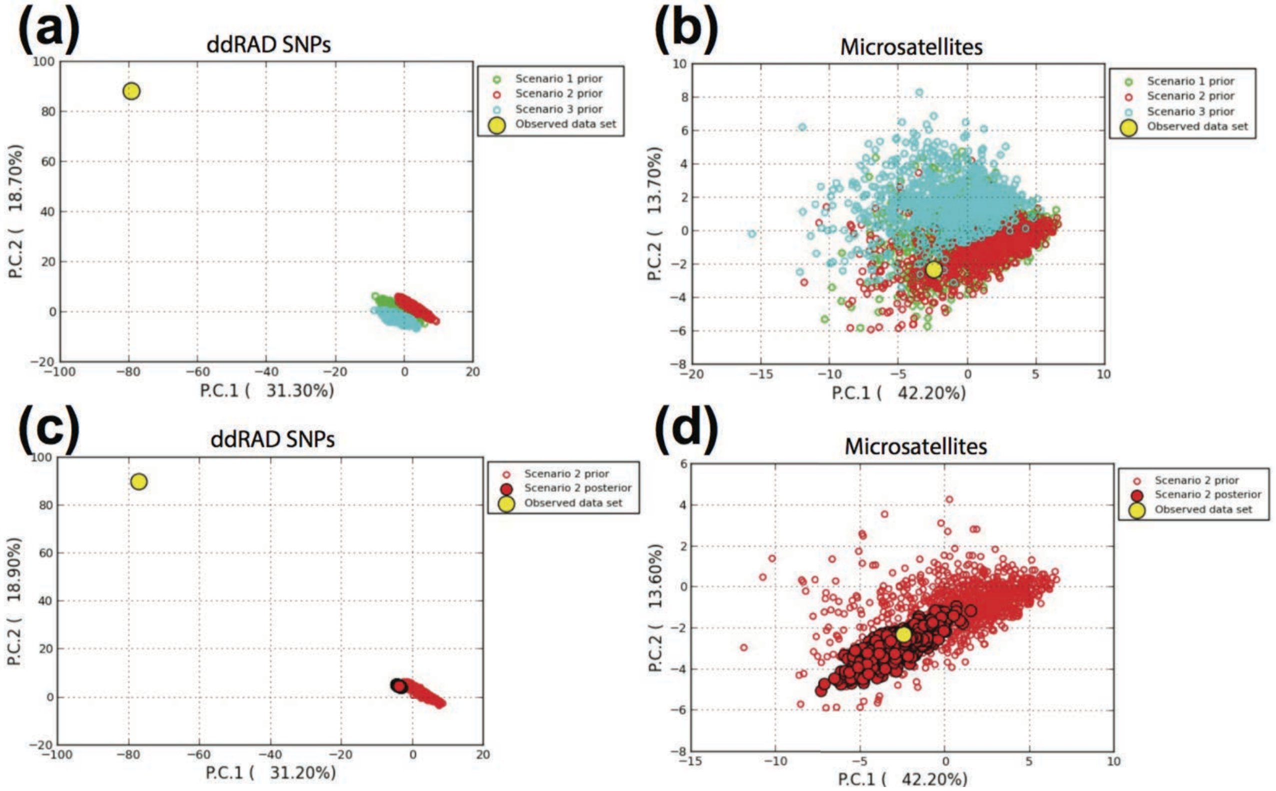


Figure S14. Principal components analysis (PCA) showing the observed data (yellow) and the ABC fit of the **(a)** ddRAD-based and **(b)** microsatellite-based model's prior distribution under each scenario colored as indicated in the legend in the upper right hand corner of each panel (Scenario 1: green, Scenario 2: red, Scenario 3: blue), and the ABC fit of the **(c)** ddRAD-based and **(d)** microsatellite-based model's posterior distributions based on the winning **Scenario 2** (red). ABC analyses was performed in DIYABC v2.0.4 (Cournuet *et al.,* 2014).

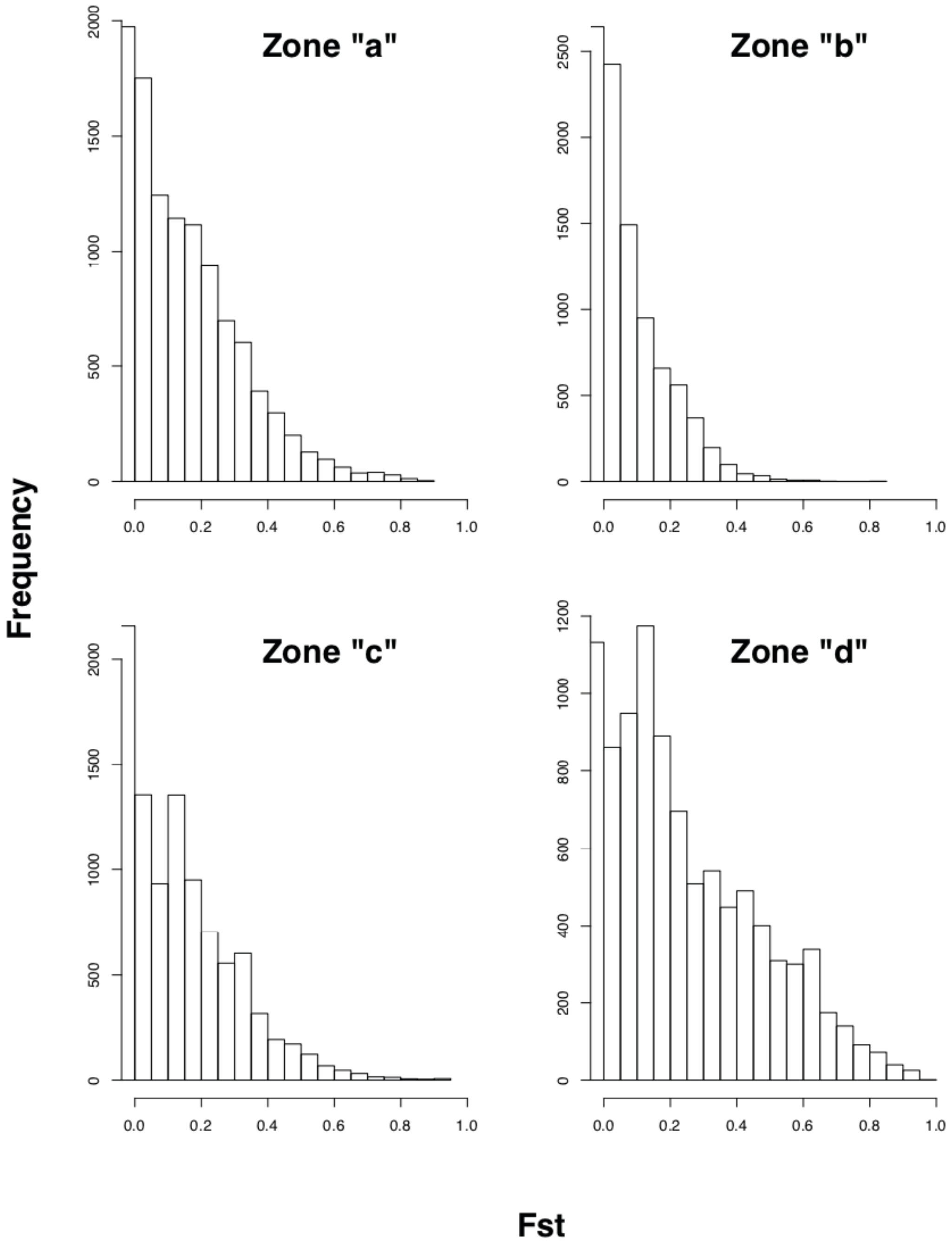


Figure S15. Histograms of ddRAD based locus-by-locus estimates of F_{ST} outliers across each of the four admixture zones completed in OutFLANK v0.2 (Whitlock and Lotterhos, 2015).

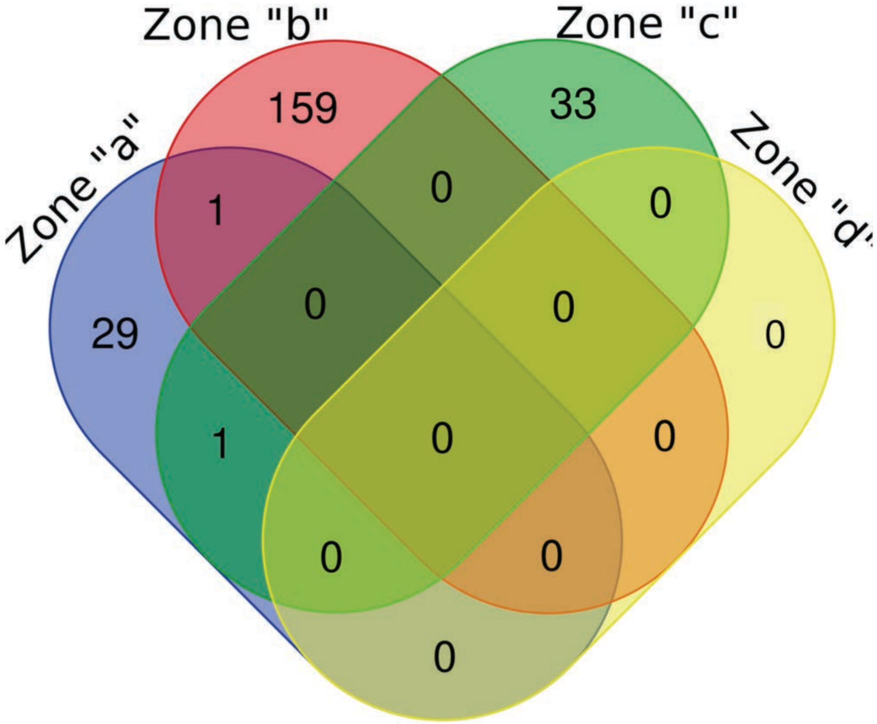


Figure S16. Overlap of SNPs with F_{ST} estimates greater than the null distribution (p-value < 0.02) across each of the four admixture zones "a" between the northwest and west, "b" between the northwest and northeast, "c" between the west and south, and "d" between the northeast and south. F_{ST} estimates and outlier tests were performed in OutFLANK v0.2 (Whitlock and Lotterhos, 2015). The Venn diagram was created using a free online bioinformatics tool http://bioinformatics.psb.ugent.be/webtools/Venn/. P-values were not corrected for multiple testing, and none of the SNPs were significant across any of the admixture zones using a more conservative empirical q-value.

Supplementary Tables

Table S1. Sample details, including sample code (numbered 1-18) indicated on the map in Figure 1 and in Table 1, sample size (N), genetic cluster (GC; northwest=NW, northeast=NE, west=W, south=W, and admixture zones "a" through "d"), sample size specific to the mtDNA analysis (N_{mtDNA}), latitude (Lat.), longitude (Long.), district name, year sampled, and statistics from VCFtools v0.1.13 (Danack *et al*, 2011) including mean read depth, and standard deviation of mean read depth (SD), and statistics from Stacks v.1.34 software (Catchen *et al* 2011) including the number of polymorphic sites (PS), number of private alleles observed (PA), the mean frequency of the major allele at each locus in this sample (P), and the sample-wide nucleotide diversity across all sites including variable and invariable positions in the 55,267 ddRAD fragments (Pi_A).

Site Code	GC	N	N_{mtDNA}	Lat.	Long.	District	Year	Depth	SD	PS	PA	P	Pi_A
01-DUK	NW	20	20	3.259	31.129	Arua	2014	46.7	289	33,976	765	0.870	0.007
02-ORB	NW	12	12	3.692	31.780	Moyo	2014	48.1	486	31,731	838	0.881	0.007
03-NGO	NW	12	12	3.669	32.591	Kitgum	2014	269.3	1446	31,119	118	0.884	0.007
04-BOL	"b"	20	14	3.287	32.793	Kitgum	2014	114.2	704	32,427	434	0.886	0.006
05-CHU	"b"	13	13	2.607	32.938	Pader	2014	50.1	433	28,641	126	0.901	0.006
06-ACA	"b"	18	17	2.270	32.521	Apac	2014	60.3	536	31,863	55	0.885	0.006
07-APU	"b"	10	10	2.079	32.676	Apac	2014	122.9	706	30,308	199	0.882	0.007
08-OCA	"b"	20	20	2.427	32.629	Apac	2014	115.6	931	31,411	70	0.883	0.006
09-UWA	"a"	15	14	2.272	31.636	Gulu	2014	156.7	930	36,395	122	0.873	0.007
10-OCU	NE	20	20	1.847	33.153	Kaberamaido	2014	105.9	785	29,529	46	0.898	0.006
11-OT	NE	14	8	1.877	33.256	Kaberamaido	2011	32.7	385	26,073	76	0.901	0.006
12-BN	"d"	12	5	1.525	33.497	Tororo	2008	56.1	62	27,962	257	0.888	0.007
13-MF	W	10	10	2.248	32.249	Gulu	2008	88.9	652	30,430	631	0.897	0.006
14-MS	W	11	11	1.630	31.691	Masindi	2011	48.9	1,156	26,381	1,025	0.890	0.006
15-KAF	"c"	20	19	1.543	32.047	Nakasongola	2015	76.2	718	28,518	418	0.889	0.006
16-JN	"c"	5	6	1.337	32.725	Nakasongola	2009	59.7	439	14,840	282	0.927	0.005
17-NB	"d"	5	3	0.592	34.059	Tororo	2011	45.9	787	13,739	388	0.933	0.004
18-WAM	S	14	14	0.369	33.345	Mayuge	2008	48.1	266	20,331	415	0.898	0.005

Table S2. Individual fly sample details: Site code, fly number, overlap (ovp.) of same individual used in the ddRAD SNP and microsatellite (*μ*sat) datasets, overlap (ovp.) of same individual used in all three datasets, the fly ID used in the SNP dataset, the fly ID used in the *μ*sat dataset, the fly ID used in the mtDNA dataset, the SNP data source, the µsat data source, and the mtDNA data source. Data sources other than this study included: [i] Beadell et al. 2010, [ii] Echodu et al 2013, [iii] Opiro et al. 2017, and [iv] Gloria-Soria et al. 2016. The final column marked † indicates the individuals used as representatives of the four genetic clusters for the MIGRAINE and ABC analysis – the northwest (NW), the northeast (NE), the west (W), and the south (S).

Site Code	Fly No.	Ovp. SNP /µsat	Ovp. SNP/ µsat/ mtDNA	ddRAD fly ID	µsat fly ID	mtDNA fly ID	SNP data source	µsat data source	mtDNA data source	†
01-DUK	1	yes	yes	DUK_14_001	DUK_14_001	DUK_14_001	this study	[iii]	this study	NW
01-DUK	2	yes	yes	DUK_14_002	DUK_14_002	DUK_14_002	this study	[iii]	[iii]	NW
01-DUK	3	yes	yes	DUK_14_003	DUK_14_003	DUK_14_003	this study	[iii]	this study	NW
01-DUK	4	yes	yes	DUK_14_007	DUK_14_007	DUK_14_007	this study	[iii]	this study	NW
01-DUK	5	yes	yes	DUK_14_011	DUK_14_011	DUK_14_011	this study	[iii]	[iii]	NW
01-DUK	6	yes	yes	DUK_14_048	DUK_14_048	DUK_14_048	this study	[iii]	[iii]	NW
01-DUK	7	yes	yes	DUK_14_056	DUK_14_056	DUK_14_056	this study	[iii]	this study	NW
01-DUK	8	yes	yes	DUK_14_388	DUK_14_388	DUK_14_388	this study	[iii]	this study	NW
01-DUK	9	yes	yes	DUK_14_389	DUK_14_389	DUK_14_389	this study	[iii]	this study	NW
01-DUK	10	yes	yes	DUK_14_518	DUK_14_518	DUK_14_518	this study	[iii]	this study	NW
01-DUK	11	yes	yes	GAN_14_086	GAN_14_086	GAN_14_086	this study	[iii]	this study	NW
01-DUK	12	yes	yes	GAN_14_101	GAN_14_101	GAN_14_101	this study	[iii]	[iii]	NW
01-DUK	13	yes	yes	GAN_14_112	GAN_14_112	GAN_14_112	this study	[iii]	[iii]	NW
01-DUK	14	yes	yes	GAN_14_136	GAN_14_136	GAN_14_136	this study	[iii]	[iii]	NW
01-DUK	15	yes	yes	GAN_14_143	GAN_14_143	GAN_14_143	this study	[iii]	[iii]	NW
01-DUK	16	yes	yes	GAN_14_148	GAN_14_148	GAN_14_148	this study	[iii]	[iii]	NW
01-DUK	17	yes	yes	GAN_14_149	GAN_14_149	GAN_14_149	this study	[iii]	[iii]	NW
01-DUK	18	yes	yes	GAN_14_252	GAN_14_252	GAN_14_252	this study	[iii]	[iii]	NW
01-DUK	19	yes	yes	GAN_14_263	GAN_14_263	GAN_14_263	this study	[iii]	[iii]	NW
01-DUK	20	yes	yes	GAN_14_281	GAN_14_281	GAN_14_281	this study	[iii]	[iii]	NW
02-ORB	21	yes	yes	ORB_14_001	ORB_14_001	ORB_14_001	this study	[iii]	[iii]	NW
02-ORB	22	yes	yes	ORB_14_006	ORB_14_006	ORB_14_006	this study	[iii]	[iii]	NW
02-ORB	23	yes	yes	ORB_14_009	ORB_14_009	ORB_14_009	this study	[iii]	[iii]	NW
02-ORB	24	yes	yes	ORB_14_010	ORB_14_010	ORB_14_010	this study	[iii]	this study	NW
02-ORB	25	yes	yes	ORB_14_011	ORB_14_011	ORB_14_011	this study	[iii]	this study	NW
02-ORB	26	yes	yes	ORB_14_012	ORB_14_012	ORB_14_012	this study	[iii]	[iii]	NW
02-ORB	27	yes	yes	ORB_14_013	ORB_14_013	ORB_14_013	this study	[iii]	[iii]	NW
02-ORB	28	yes	yes	ORB_14_014	ORB_14_014	ORB_14_014	this study	[iii]	this study	NW
02-ORB	29	yes	yes	ORB_14_015	ORB_14_015	ORB_14_015	this study	[iii]	[iii]	NW
02-ORB	30	yes	yes	ORB_14_021	ORB_14_021	ORB_14_021	this study	[iii]	this study	NW
02-ORB	31	yes	yes	ORB_14_022	ORB_14_022	ORB_14_022	this study	[iii]	[iii]	NW
02-ORB	32	yes	yes	ORB_14_024	ORB_14_024	ORB_14_024	this study	[iii]	this study	NW
03-NGO	33	yes	yes	NGO_14_008	NGO_14_008	NGO_14_008	this study	[iii]	this study	

03-NGO	34	yes	yes	NGO_14_009	NGO_14_009	NGO_14_009	this study	[iii]	[iii]	
03-NGO	35	yes	yes	NGO_14_013	NGO_14_013	NGO_14_013	this study	[iii]	[iii]	
03-NGO	36	yes	yes	NGO_14_014	NGO_14_014	NGO_14_014	this study	[iii]	this study	
03-NGO	37	yes	yes	NGO_14_015	NGO_14_015	NGO_14_015	this study	[iii]	this study	
03-NGO	38	yes	yes	NGO_14_030	NGO_14_030	NGO_14_030	this study	[iii]	this study	
03-NGO	39	yes	yes	NGO_14_050	NGO_14_050	NGO_14_050	this study	[iii]	this study	
03-NGO	40	yes	yes	NGO_14_057	NGO_14_057	NGO_14_057	this study	[iii]	[iii]	
03-NGO	41	yes	yes	NGO_14_062	NGO_14_062	NGO_14_062	this study	[iii]	this study	
03-NGO	42	yes	yes	NGO_14_063	NGO_14_063	NGO_14_063	this study	[iii]	this study	
03-NGO	43	yes	yes	NGO_14_065	NGO_14_065	NGO_14_065	this study	[iii]	this study	
03-NGO	44	yes	yes	NGO_14_072	NGO_14_072	NGO_14_072	this study	[iii]	this study	
04-BOL	45	yes	yes	BOL_14_040	BOL_14_040	BOL_14_040	this study	[iii]	this study	
04-BOL	46	yes	yes	BOL_14_064	BOL_14_064	BOL_14_064	this study	[iii]	[iii]	
04-BOL	47	yes	no	BOL_14_065	BOL_14_060	N/A	this study	this study	N/A	
04-BOL	48	yes	no	BOL_14_083	BOL_14_083	N/A	this study	[iii]	N/A	
04-BOL	49	yes	yes	BOL_14_086	BOL_14_086	BOL_14_086	this study	[iii]	this study	
04-BOL	50	yes	yes	BOL_14_089	BOL_14_089	BOL_14_089	this study	[iii]	this study	
04-BOL	51	yes	yes	BOL_14_112	BOL_14_112	BOL_14_112	this study	[iii]	[iii]	
04-BOL	52	yes	yes	BOL_14_113	BOL_14_113	BOL_14_113	this study	[iii]	[iii]	
04-BOL	53	yes	yes	BOL_14_144	BOL_14_144	BOL_14_144	this study	[iii]	[iii]	
04-BOL	54	yes	yes	BOL_14_224	BOL_14_224	BOL_14_224	this study	[iii]	this study	
04-BOL	55	yes	yes	KTC_14_005	KTC_14_005	KTC_14_005	this study	[iii]	[iii]	
04-BOL	56	yes	no	KTC_14_011	KTC_14_011	N/A	this study	[iii]	N/A	
04-BOL	57	yes	no	KTC_14_012	KTC_14_012	N/A	this study	[iii]	N/A	
04-BOL	58	yes	yes	KTC_14_016	KTC_14_016	KTC_14_016	this study	[iii]	[iii]	
04-BOL	59	yes	yes	KTC_14_021	KTC_14_021	KTC_14_021	this study	[iii]	this study	
04-BOL	60	yes	no	KTC_14_023	KTC_14_023	N/A	this study	[iii]	N/A	
04-BOL	61	yes	no	KTC_14_116	KTC_15_116	N/A	this study	this study	N/A	
04-BOL	62	yes	yes	KTC_14_118	KTC_14_118	KTC_14_118	this study	[iii]	this study	
04-BOL	63	yes	yes	KTC_14_234	KTC_14_234	KTC_14_234	this study	[iii]	this study	
04-BOL	64	yes	yes	KTC_14_255	KTC_14_255	KTC_14_255	this study	[iii]	this study	
05-CHU	65	yes	yes	CHU_14_004	CHU_14_004	CHU_14_004	this study	[iii]	[iii]	
05-CHU	66	yes	yes	CHU_14_010	CHU_14_010	CHU_14_010	this study	[iii]	this study	
05-CHU	67	yes	yes	CHU_14_021	CHU_14_021	CHU_14_021	this study	[iii]	this study	
05-CHU	68	yes	yes	CHU_14_032	CHU_14_032	CHU_14_032	this study	[iii]	this study	
05-CHU	69	yes	yes	CHU_14_045	CHU_14_045	CHU_14_045	this study	[iii]	this study	
05-CHU	70	yes	yes	CHU_14_050	CHU_14_050	CHU_14_050	this study	[iii]	[iii]	
05-CHU	71	yes	yes	CHU_14_053	CHU_14_053	CHU_14_053	this study	[iii]	this study	
05-CHU	72	yes	yes	CHU_14_059	CHU_14_059	CHU_14_059	this study	[iii]	[iii]	
05-CHU	73	yes	yes	CHU_14_068	CHU_14_068	CHU_14_068	this study	[iii]	[iii]	
05-CHU	74	yes	yes	CHU_14_086	CHU_14_086	CHU_14_086	this study	[iii]	this study	
05-CHU	75	yes	yes	CHU_14_095	CHU_14_095	CHU_14_095	this study	[iii]	this study	
05-CHU	76	yes	yes	CHU_14_099	CHU_14_099	CHU_14_099	this study	[iii]	this study	
05-CHU	77	yes	yes	CHU_14_103	CHU_14_103	CHU_14_103	this study	[iii]	[iii]	
06-ACA	78	yes	yes	ACA_14_1010	ACA_14_1010	ACA_14_1010	this study	[iii]	this study	"b"
06-ACA	79	yes	yes	ACA_14_1011	ACA_14_1011	ACA_14_1011	this study	[iii]	[iii]	"b"
06-ACA	80	yes	yes	ACA_14_1012	ACA_14_1012	ACA_14_1012	this study	[iii]	this study	"b"
06-ACA	81	yes	yes	ACA_14_1026	ACA_14_1026	ACA_14_1026	this study	[iii]	[iii]	"b"
06-ACA	82	yes	yes	ACA_14_1030	ACA_14_1030	ACA_14_1030	this study	[iii]	[iii]	"b"

06-ACA	83	yes	yes	ACA_14_1031	ACA_14_1031	ACA_14_1031	this study	[iii]	[iii]	"b"
06-ACA	84	yes	yes	ACA_14_1109	ACA_14_1109	ACA_14_1109	this study	[iii]	this study	"b"
06-ACA	85	yes	yes	ACA_14_1124	ACA_14_1124	ACA_14_1124	this study	[iii]	[iii]	"b"
06-ACA	86	yes	yes	ACA_14_1125	ACA_14_1125	ACA_14_1125	this study	[iii]	[iii]	"b"
06-ACA	87	yes	yes	ACA_14_589	ACA_14_589	ACA_14_589	this study	[iii]	this study	"b"
06-ACA	88	yes	no	ACA_14_654	ACA_14_654	N/A	this study	[iii]	N/A	"b"
06-ACA	89	yes	yes	ACA_14_777	ACA_14_777	ACA_14_777	this study	[iii]	this study	"b"
06-ACA	90	yes	yes	ACA_14_819	ACA_14_819	ACA_14_819	this study	[iii]	this study	"b"
06-ACA	91	yes	yes	ACA_14_828	ACA_14_828	ACA_14_828	this study	[iii]	this study	"b"
06-ACA	92	yes	yes	ACA_14_888	ACA_14_888	ACA_14_888	this study	[iii]	this study	"b"
06-ACA	93	yes	yes	ACA_14_898	ACA_14_898	ACA_14_898	this study	[iii]	this study	"b"
06-ACA	94	yes	yes	ACA_14_994	ACA_14_994	ACA_14_994	this study	[iii]	this study	"b"
06-ACA	95	yes	yes	ACA_14_999	ACA_14_999	ACA_14_999	this study	[iii]	this study	"b"
07-APU	96	yes	yes	APU_14_644	APU_14_644	APU_14_644	this study	[iii]	[iii]	"b"
07-APU	97	yes	yes	APU_14_652	APU_14_652	APU_14_652	this study	[iii]	[iii]	"b"
07-APU	98	yes	yes	APU_14_667	APU_14_667	APU_14_667	this study	[iii]	[iii]	"b"
07-APU	99	yes	yes	APU_14_683	APU_14_683	APU_14_683	this study	[iii]	[iii]	"b"
07-APU	100	yes	yes	APU_14_694	APU_14_694	APU_14_694	this study	[iii]	[iii]	"b"
07-APU	101	no	no	APU_14_696	APU_14_642	APU_14_696	this study	this study	this study	"b"
07-APU	102	yes	yes	APU_14_697	APU_14_697	APU_14_697	this study	[iii]	[iii]	"b"
07-APU	103	no	no	APU_14_705	APU_15_705	APU_14_705	this study	this study	this study	"b"
07-APU	104	no	no	APU_14_706	APU_15_706	APU_14_706	this study	this study	this study	"b"
07-APU	105	yes	yes	APU_14_717	APU_14_717	APU_14_717	this study	[iii]	[iii]	"b"
08-OCA	106	yes	yes	OCA_14_032	OCA_14_032	OCA_14_032	this study	[iii]	this study	"b"
08-OCA	107	yes	yes	OCA_14_062	OCA_14_062	OCA_14_062	this study	[iii]	this study	"b"
08-OCA	108	yes	yes	OCA_14_1096	OCA_14_1096	OCA_14_1096	this study	[iii]	this study	"b"
08-OCA	109	yes	yes	OCA_14_1102	OCA_14_1102	OCA_14_1102	this study	[iii]	this study	"b"
08-OCA	110	yes	yes	OCA_14_138	OCA_14_138	OCA_14_138	this study	[iii]	this study	"b"
08-OCA	111	yes	yes	OCA_14_171	OCA_14_171	OCA_14_171	this study	[iii]	this study	"b"
08-OCA	112	yes	yes	OCA_14_206	OCA_14_206	OCA_14_206	this study	[iii]	this study	"b"
08-OCA	113	yes	yes	OCA_14_242	OCA_14_242	OCA_14_242	this study	[iii]	this study	"b"
08-OCA	114	yes	yes	OCA_14_299	OCA_14_299	OCA_14_299	this study	[iii]	this study	"b"
08-OCA	115	yes	yes	OCA_14_402	OCA_14_402	OCA_14_402	this study	[iii]	this study	"b"
08-OCA	116	yes	yes	OCA_14_711	OCA_14_711	OCA_14_711	this study	[iii]	this study	"b"
08-OCA	117	yes	yes	OCA_14_713	OCA_14_713	OCA_14_713	this study	[iii]	this study	"b"
08-OCA	118	yes	yes	OCA_14_715	OCA_14_715	OCA_14_715	this study	[iii]	this study	"b"
08-OCA	119	yes	yes	OCA_14_733	OCA_14_733	OCA_14_733	this study	[iii]	this study	"b"
08-OCA	120	yes	yes	OCA_14_737	OCA_14_737	OCA_14_737	this study	[iii]	this study	"b"
08-OCA	121	yes	yes	OCA_14_741	OCA_14_741	OCA_14_741	this study	[iii]	this study	"b"
08-OCA	122	yes	yes	OCA_14_755	OCA_14_755	OCA_14_755	this study	[iii]	this study	"b"
08-OCA	123	yes	yes	OCA_14_758	OCA_14_758	OCA_14_758	this study	[iii]	this study	"b"
08-OCA	124	yes	yes	OCA_14_776	OCA_14_776	OCA_14_776	this study	[iii]	this study	"b"
08-OCA	125	yes	yes	OCA_14_784	OCA_14_784	OCA_14_784	this study	[iii]	this study	"b"
09-UWA	126	yes	yes	UWA_14_001	UWA_14_001	UWA_14_001	this study	[iii]	this study	
09-UWA	127	yes	yes	UWA_14_004	UWA_14_004	UWA_14_004	this study	[iii]	this study	
09-UWA	128	yes	yes	UWA_14_005	UWA_14_005	UWA_14_005	this study	[iii]	this study	
09-UWA	129	yes	yes	UWA_14_009	UWA_14_009	UWA_14_009	this study	[iii]	[iii]	
09-UWA	130	yes	yes	UWA_14_011	UWA_14_011	UWA_14_011	this study	[iii]	[iii]	
09-UWA	131	yes	yes	UWA_14_014	UWA_14_014	UWA_14_014	this study	[iii]	[iii]	

09-UWA	132	yes	yes	UWA_14_018	UWA_14_018	UWA_14_018	this study	[iii]	this study	
09-UWA	133	yes	yes	UWA_14_034	UWA_14_034	UWA_14_034	this study	[iii]	this study	
09-UWA	134	yes	yes	UWA_14_036	UWA_14_036	UWA_14_036	this study	[iii]	[iii]	
09-UWA	135	yes	yes	UWA_14_037	UWA_14_037	UWA_14_037	this study	[iii]	[iii]	
09-UWA	136	yes	yes	UWA_14_039	UWA_14_039	UWA_14_039	this study	[iii]	this study	
09-UWA	137	yes	yes	UWA_14_122	UWA_14_122	UWA_14_122	this study	[iii]	this study	
09-UWA	138	no	no	UWA_14_133	UWA_14_002	N/A	this study	this study	N/A	
09-UWA	139	yes	yes	UWA_14_311	UWA_14_311	UWA_14_311	this study	[iii]	this study	
09-UWA	140	yes	yes	UWA_14_422	UWA_14_422	UWA_14_422	this study	[iii]	this study	
10-OCU	141	yes	yes	OCU_14_012	OCU_14_012	OCU_14_012	this study	[iii]	this study	NE
10-OCU	142	yes	yes	OCU_14_021	OCU_14_021	OCU_14_021	this study	[iii]	this study	NE
10-OCU	143	yes	yes	OCU_14_161	OCU_14_161	OCU_14_161	this study	[iii]	this study	NE
10-OCU	144	yes	yes	OCU_14_199	OCU_14_199	OCU_14_199	this study	[iii]	[iii]	NE
10-OCU	145	yes	yes	OCU_14_230	OCU_14_230	OCU_14_230	this study	[iii]	[iii]	NE
10-OCU	146	yes	yes	OCU_14_262	OCU_14_262	OCU_14_262	this study	[iii]	this study	NE
10-OCU	147	yes	yes	OCU_14_284	OCU_14_284	OCU_14_284	this study	[iii]	this study	NE
10-OCU	148	yes	yes	OCU_14_427	OCU_14_427	OCU_14_427	this study	[iii]	this study	NE
10-OCU	149	yes	yes	OCU_14_431	OCU_14_431	OCU_14_431	this study	[iii]	this study	NE
10-OCU	150	yes	yes	OCU_14_432	OCU_14_432	OCU_14_432	this study	[iii]	this study	NE
10-OCU	151	yes	yes	OCU_14_446	OCU_14_446	OCU_14_446	this study	[iii]	this study	NE
10-OCU	152	yes	yes	OCU_14_457	OCU_14_457	OCU_14_457	this study	[iii]	this study	NE
10-OCU	153	yes	yes	OCU_14_468	OCU_14_468	OCU_14_468	this study	[iii]	this study	NE
10-OCU	154	yes	yes	OCU_14_482	OCU_14_482	OCU_14_482	this study	[iii]	[iii]	NE
10-OCU	155	yes	yes	OCU_14_512	OCU_14_512	OCU_14_512	this study	[iii]	[iii]	NE
10-OCU	156	yes	yes	OCU_14_515	OCU_14_515	OCU_14_515	this study	[iii]	[iii]	NE
10-OCU	157	yes	yes	OCU_14_534	OCU_14_534	OCU_14_534	this study	[iii]	this study	NE
10-OCU	158	yes	yes	OCU_14_618	OCU_14_618	OCU_14_618	this study	[iii]	this study	NE
10-OCU	159	yes	yes	OCU_14_640	OCU_14_640	OCU_14_640	this study	[iii]	this study	NE
10-OCU	160	yes	yes	OCU_14_660	OCU_14_660	OCU_14_660	this study	[iii]	this study	NE
11-OT	161	no	no	OT_11_047	OC_12_001	N/A	[iv]	[iii]	N/A	NE
11-OT	162	no	no	OT_11_048	OC_12_002	OT_11_048	[iv]	[iii]	this study	NE
11-OT	163	no	no	OT_11_049	OC_12_003	OT_11_049	[iv]	[iii]	this study	NE
11-OT	164	no	no	OT_11_052	OC_12_004	OT_11_052	[iv]	[iii]	this study	NE
11-OT	165	no	no	OT_11_054	OC_12_005	OT_11_054	[iv]	[iii]	this study	NE
11-OT	166	no	no	OT_11_055	OC_12_006	N/A	[iv]	[iii]	N/A	NE
11-OT	167	no	no	OT_11_072	OC_12_007	N/A	[iv]	[iii]	N/A	NE
11-OT	168	no	no	OT_11_073	OC_12_008	OT_11_073	[iv]	[iii]	this study	NE
11-OT	169	no	no	OT_11_079	OC_12_009	OT_11_079	[iv]	[iii]	this study	NE
11-OT	170	no	no	OT_11_083	OC_12_010	OT_11_083	[iv]	[iii]	this study	NE
11-OT	171	no	no	OT_11_086	OC_12_011	N/A	[iv]	[iii]	N/A	NE
11-OT	172	no	no	OT_11_094	OC_12_012	N/A	[iv]	[iii]	N/A	NE
11-OT	173	no	no	OT_11_098	OC_12_013	N/A	[iv]	[iii]	N/A	NE
11-OT	174	no	no	OT_11_100	OC_12_014	OT_11_100	[iv]	[iii]	this study	NE
12-BN	175	yes	no	BN_08_3-25	BN_08_3-25	N/A	[iv]	[i], 5 loci this study	N/A	
12-BN	176	yes	yes	BN_08_3-26	BN_08_3-26	BN_08_3-26	[iv]	[i], 5 loci this study	[i]	
12-BN	177	yes	yes	BN_08_6-13	BN_08_6-13	BN_08_6-13	[iv]	[i], 5 loci this study	[i]	
12-BN	178	yes	yes	BN_08_6-15	BN_08_6-15	BN_08_6-15	[iv]	[i], 5 loci this study	[i]	
12-BN	179	yes	yes	BN_08_6-17	BN_08_6-17	BN_08_6-17	[iv]	[i], 5 loci this study	[i]	
12-BN	180	yes	no	BN_08_7-4	BN_08_7-4	N/A	[iv]	[i], 5 loci this study	N/A	

12-BN	181	yes	no	BN_08_7-6	BN_08_7-6	N/A	[iv]	[i], 5 loci this study	N/A	
12-BN	182	yes	no	BN_08_7-8	BN_08_7-8	N/A	[iv]	[i], 5 loci this study	N/A	
12-BN	183	yes	no	BN_08_8-1	BN_08_8-1	N/A	[iv]	[i], 5 loci this study	N/A	
12-BN	184	yes	yes	BN_08_8-3	BN_08_8-3	BN_08_8-3	[iv]	[i], 5 loci this study	[i]	
12-BN	185	yes	no	BN_08_8-5	BN_08_8-5	N/A	[iv]	[i], 5 loci this study	N/A	
12-BN	186	yes	no	BN_08_8-7	BN_08_8-7	N/A	[iv]	[i], 5 loci this study	N/A	
13-MF	187	yes	yes	MF_09_001	MF_09_001	MF_09_001	this study	[i], 5 loci this study	[i]	W
13-MF	188	yes	yes	MF_09_002	MF_09_002	MF_09_002	this study	[i], 5 loci this study	[i]	W
13-MF	189	yes	yes	MF_09_003	MF_09_003	MF_09_003	this study	[i], 5 loci this study	[i]	W
13-MF	190	yes	yes	MF_09_004	MF_09_004	MF_09_004	this study	[i], 5 loci this study	[i]	W
13-MF	191	yes	yes	MF_09_005	MF_09_005	MF_09_005	this study	[i], 5 loci this study	[i]	W
13-MF	192	yes	yes	MF_09_006	MF_09_006	MF_09_006	this study	[i], 5 loci this study	[i]	W
13-MF	193	yes	yes	MF_09_007	MF_09_007	MF_09_007	this study	[i], 5 loci this study	[i]	W
13-MF	194	yes	yes	MF_09_008	MF_09_008	MF_09_008	this study	[i], 5 loci this study	[i]	W
13-MF	195	yes	yes	MF_09_009	MF_09_009	MF_09_009	this study	[i], 5 loci this study	[i]	W
13-MF	196	yes	yes	MF_09_010	MF_09_010	MF_09_010	this study	[i], 5 loci this study	[i]	W
14-MS	197	yes	yes	MS_11_001	MS_11_001	MS_11_001	this study	[i], [iii]	[iii]	W
14-MS	198	no	no	MS_11_004	MS_11_002	MS_11_004	this study	this study	this study	W
14-MS	199	no	no	MS_11_006	MS_11_011	MS_11_006	this study	this study	this study	W
14-MS	200	no	no	MS_11_012	MS_11_020	MS_11_012	this study	[ii], [iii]	[iii]	W
14-MS	201	no	no	MS_11_013	MS_11_022	MS_11_013	this study	this study	this study	W
14-MS	202	no	no	MS_11_014	MS_11_023	MS_11_014	this study	this study	this study	W
14-MS	203	no	no	MS_11_015	MS_11_028	MS_11_015	this study	this study	this study	W
14-MS	204	yes	yes	MS_11_019	MS_11_019	MS_11_019	this study	[ii], [iii]	[iii]	W
14-MS	205	yes	yes	MS_11_021	MS_11_021	MS_11_021	this study	[ii], [iii]	[iii]	W
14-MS	206	yes	yes	MS_11_024	MS_11_024	MS_11_024	this study	[ii], [iii]	[iii]	W
14-MS	207	yes	yes	MS_11_031	MS_11_031	MS_11_031	this study	[ii], [iii]	[iii]	W
15-KAF	208	yes	yes	KAF_15_006	KAF_15_006	KAF_15_006	this study	[iii]	this study	W
15-KAF	209	yes	yes	KAF_15_007	KAF_15_007	KAF_15_007	this study	[iii]	this study	W
15-KAF	210	yes	no	KAF_15_026	KAF_15_026	N/A	this study	[iii]	N/A	W
15-KAF	211	yes	yes	KAF_15_038	KAF_15_038	KAF_15_038	this study	[iii]	this study	W
15-KAF	212	yes	yes	KAF_15_047	KAF_15_047	KAF_15_047	this study	[iii]	this study	W
15-KAF	213	yes	yes	KAF_15_054	KAF_15_054	KAF_15_054	this study	[iii]	this study	W
15-KAF	214	yes	yes	KAF_15_069	KAF_15_069	KAF_15_069	this study	[iii]	this study	W
15-KAF	215	yes	yes	KAF_15_079	KAF_15_079	KAF_15_079	this study	[iii]	this study	W
15-KAF	216	yes	yes	KAF_15_080	KAF_15_080	KAF_15_080	this study	[iii]	this study	W
15-KAF	217	yes	yes	KAF_15_089	KAF_15_089	KAF_15_089	this study	[iii]	this study	W
15-KAF	218	yes	yes	KAF_15_095	KAF_15_095	KAF_15_095	this study	[iii]	this study	W
15-KAF	219	yes	yes	KAF_15_113	KAF_15_113	KAF_15_113	this study	[iii]	this study	W
15-KAF	220	yes	yes	KAF_15_143	KAF_15_143	KAF_15_143	this study	[iii]	this study	W
15-KAF	221	yes	yes	KAF_15_144	KAF_15_144	KAF_15_144	this study	[iii]	this study	W
15-KAF	222	yes	yes	KAF_15_157	KAF_15_157	KAF_15_157	this study	[iii]	this study	W
15-KAF	223	yes	yes	KAF_15_161	KAF_15_161	KAF_15_161	this study	[iii]	this study	W
15-KAF	224	yes	yes	KAF_15_165	KAF_15_165	KAF_15_165	this study	[iii]	this study	W
15-KAF	225	yes	yes	KAF_15_173	KAF_15_173	KAF_15_173	this study	[iii]	this study	W
15-KAF	226	yes	yes	KAF_15_196	KAF_15_196	KAF_15_196	this study	[iii]	this study	W
15-KAF	227	yes	yes	KAF_15_210	KAF_15_210	KAF_15_210	this study	[iii]	this study	W
16-JN	228	no	no	JN_09_61	JN_09_61	JN_09_54	this study	[i], 5 loci this study	[i]	
16-JN	229	yes	yes	JN_09_62	JN_09_62	JN_09_62	this study	[i], 5 loci this study	[i]	

16-JN	230	yes	yes	JN_09_66	JN_09_66	JN_09_66	this study	[i], 5 loci this study	[i]	
16-JN	231	yes	yes	JN_09_68	JN_09_68	JN_09_68	this study	[i], 5 loci this study	[i]	
16-JN	234	yes	yes	JN_09_79	JN_09_79	JN_09_79	this study	[i], 5 loci this study	[i]	
17-NB	235	yes	no	NB_11_019	NB_11_019	N/A	[iv]	[ii], 5 loci this	N/A	
17-NB	236	no	no	NB_11_031	NB_11_030	NB_11_031	[iv]	[ii], 5 loci this	this study	
17-NB	237	no	no	NB_11_034	NB_11_046	NB_11_034	[iv]	[ii], 5 loci this	this study	
17-NB	238	no	no	NB_11_040	NB_11_047	NB_11_040	[iv]	[ii], 5 loci this	this study	
17-NB	239	no	no	NB_11_059	NB_11_059	N/A	[iv]	[ii], 5 loci this	N/A	
18-WAM	240	yes	yes	WAM_16_022	WAM_16_022	WAM_16_022	this study	this study	this study	S
18-WAM	241	yes	yes	WAM_16_023	WAM_16_023	WAM_16_023	this study	this study	this study	S
18-WAM	242	yes	yes	WAM_16_026	WAM_16_026	WAM_16_026	this study	this study	this study	S
18-WAM	243	yes	yes	WAM_16_033	WAM_16_033	WAM_16_033	this study	this study	this study	S
18-WAM	244	yes	yes	WAM_16_034	WAM_16_034	WAM_16_034	this study	this study	this study	S
18-WAM	245	yes	yes	WAM_16_041	WAM_16_041	WAM_16_041	this study	this study	this study	S
18-WAM	246	yes	yes	WAM_16_052	WAM_16_052	WAM_16_052	this study	this study	this study	S
18-WAM	247	yes	yes	WAM_16_084	WAM_16_084	WAM_16_084	this study	this study	this study	S
18-WAM	248	yes	yes	WAM_16_112	WAM_16_112	WAM_16_112	this study	this study	this study	S
18-WAM	249	yes	yes	WAM_16_113	WAM_16_113	WAM_16_113	this study	this study	this study	S
18-WAM	250	yes	yes	WAM_16_163	WAM_16_163	WAM_16_163	this study	this study	this study	S
18-WAM	251	yes	yes	WAM_16_172	WAM_16_172	WAM_16_172	this study	this study	this study	S
18-WAM	252	yes	yes	WAM_16_186	WAM_16_186	WAM_16_186	this study	this study	this study	S
18-WAM	253	yes	yes	WAM_16_233	WAM_16_233	WAM_16_233	this study	this study	this study	S

Table S3. ABC analysis priors used in DIYABC v2.0.4 (Cournuet *et al.,* 2014). **(a)** Global priors applied to all ABC analysis. For the ddRAD SNPs, the only method available was a simplified and efficient algorithm that does not require or accept any parameterization of the mutation model (Cornuet et al. 2014) beyond a minor allele frequency (MAF), which we restricted as little as possible to the MAF that equates to 0.05 (DIYABC flag <MAF=hudson>). For the microsatellites, we used the default Generalized Stepwise Mutation Model (Estoup et al., 2002) with a mutation rate of 1E-3 to 1E-4, and a starting parameter of the geometric distribution (*P*) of 0.22 (Table S3). For all analyses, N_e priors were chosen to be consistent with those made in previous studies based on microsatellite data (Beadell et al. 2010; Echodu et al. 2011; Opiro et al. 2017). Time priors (minimum and maximum time before the time of sampling in years) for the serial preliminary analyses **(b)** 1, **(c)** 2, **(d)** and 3, were specified with increasing specificity that matched the biogeographic history of *G. f. fuscipes* (see Figure 3) until the posterior estimates were not truncated by zero (see Figure S13). Results from this preliminary analysis were then used to set time priors for **(e)** the final ABC analysis.

(a) Global priors

	Min	**Max**	**Mean**	**Model**	**Scenario**
ddRAD SNPs	0.05	N/A	N/A	Hudson (2002)	All
Microsatellites	1E-4	1E-3	5E-4	GSM, mean *P=0.22*	All
Ne	100	30,000	N/A	N/A	All
Ne during bottleneck	10	5,000	N/A	N/A	All

(b) Time priors – preliminary analysis 1	**Prior Min (y)**	**Prior Max (y)**	**Conditions**	**Posterior Median (y)**	**Scenario**
t1/t2	0	650,000	None	0	All
t3	0	650,000	t3 > t1/t2	399	All
t4	0	650,000	t4 > t3	306,250	All

(c) Time priors – preliminary analysis 2	**Prior Min (y)**	**Prior Max (y)**	**Conditions**	**Posterior Median (y)**	**Scenario**
t1/t2	0	650,000	None	49	All
t3	0	650,000	t3 > t1/t2	5,263	All
t4	340,000	650,000	t4 > t3	426,250	All

(d) Time priors – preliminary analysis 3	**Prior Min (y)**	**Prior Max (y)**	**Conditions**	**Posterior Median (y)**	**Scenario**
t1/t2	0	650,000	None	50	All
t3	20,000	650,000	t3 > t1/t2	21,750	All
t4	340,000	650,000	t4 > t3	373,750	All

(e) Time priors – final analysis	**Prior Min (y)**	**Prior Max (y)**	**Scenario**
t1	5	135	1 and 2
t2	10,000	20,000	1 and 3
t3	20,000	40,000	All
t4	340,000	460,000	All
bd (bottleneck duration)	1.25	5,000	3

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Table S4. ddRAD SNP and microsatellite (µsat) *p*-values corrected for multiple testing of the inbreeding coefficient F_{IS} (Nei 1987) using the Benjamini-Hochberg (BH) method (Benjamini and Hochberg, 1995) and the Bonferroni (BF) method (Dunn 1961) in the R package "stats" (R Core Team, 2016). For 55,267 ddRAD SNPs (ddRAD), *p*-values are based on bootstrap (*n* = 1,000) sampling in the R package "hierFSTAT" (Goudet and Jombart, 2015). For the 16 microsatellites (µsat), *p*-values are based on 1000 randomizations in FSTAT.

Sample	**ddRAD F_{IS}**	**ddRAD *p*-value**	**ddRAD BH**	**ddRAD BF**	**µsat F_{IS}**	**µsat *p*-value**	**µsat BH**	**µsat BF**
01-DUK	-0.169	0.0001	0.0001	0.0018	0.123	0.0003	0.0011	0.0054
02-ORB	0.081	0.0001	0.0001	0.0018	0.180	0.0002	0.0011	0.0036
03-NGO	0.206	0.0001	0.0001	0.0018	0.061	0.0878	0.1317	1.0000
04-BOL	-0.104	0.0001	0.0001	0.0018	0.096	0.0031	0.0080	0.0558
05-CHU	0.095	0.0001	0.0001	0.0018	0.024	0.2859	0.3676	1.0000
06-ACA	-0.071	0.0001	0.0001	0.0018	0.144	0.0002	0.0011	0.0036
07-APU	-0.115	0.0001	0.0001	0.0018	0.117	0.0184	0.0368	0.3312
08-OCA	-0.052	0.0001	0.0001	0.0018	0.120	0.0007	0.0021	0.0126
09-UWA	0.190	0.0001	0.0001	0.0018	0.016	0.3410	0.3836	1.0000
10-OCU	0.000	1.0000	1.0000	1.0000	0.135	0.0003	0.0011	0.0054
11-OT	0.009	0.0010	0.0011	0.0180	-0.068	0.0773	0.1265	1.0000
12-BN	0.059	0.0001	0.0001	0.0018	0.211	0.0002	0.0011	0.0036
13-MF	0.063	0.0001	0.0001	0.0018	0.122	0.0104	0.0234	0.1872
14-MS	0.230	0.0001	0.0001	0.0018	0.040	0.2377	0.3291	1.0000
15-KAF	0.212	0.0001	0.0001	0.0018	0.070	0.0410	0.0738	0.7380
16-JN	0.031	0.0001	0.0001	0.0018	0.043	0.3378	0.3836	1.0000
17-NB	0.003	1.0000	1.0000	1.0000	0.012	0.5208	0.5208	1.0000
18-WAM	-0.035	0.0001	0.0001	0.0018	0.006	0.4696	0.4972	1.0000
Albert Nile	-0.050	0.0001	0.0001	0.0007	0.145	0.0004	0.0006	0.0028
Achwa River	0.055	0.0001	0.0001	0.0007	0.082	0.0004	0.0006	0.0028
Okole River	0.014	0.0001	0.0001	0.0007	0.175	0.0004	0.0006	0.0028
Lake Kyoga	0.067	0.0001	0.0001	0.0007	0.144	0.0004	0.0006	0.0028
Lake Albert	0.186	0.0001	0.0001	0.0007	0.129	0.0004	0.0006	0.0028
Kafu River	0.212	0.0001	0.0001	0.0007	0.070	0.0411	0.0480	0.2877
Lake Victoria	0.101	0.0001	0.0001	0.0007	0.048	0.1308	0.1308	0.9156

Table S5. Results from analysis of molecular variance (AMOVA) for **(A)** the 2,046 ddRAD SNPs >95% genotyping rate, **(B)** 16 microsatellite loci, and **(C)** a 490 bp mtDNA COI-COII gene fragment, showing the degrees of freedom (d.f.), the sum of squares, total variance components, percentage of variation explained, fixation index and p-value for three levels (among watersheds, among sites within watersheds, and within sites). The AMOVA was performed with the POPPR R library for the ddRAD SNP data set and ARLEQUIN v3.5 (Excoffer *et al*, 2010) for the microsatellite and mtDNA data sets.

ddRAD SNPs source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Index	P-value
Among watersheds	6	97,015.69	204.17	68.9%	0.325	<0.001
Among sites within watersheds	11	19,717.77	39.29	12.9%	0.089	<0.001
Within sites	233	176,897.29	61.88	20.3%	0.053	<0.001
Total	501	453,130.25	940.79			

Microsatellites source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Index	P-value
Among watersheds	6	166.92	0.239	7.1%	0.071	<0.001
Among sites within watersheds	11	111.45	0.277	8.3%	0.089	<0.001
Within sites	484	1371.42	2.833	84.6%	0.154	<0.001
Total	501	1649.79	3.349			

mtDNA source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Index	P-value
Among watersheds	6	98.84	0.348	28.2%	0.282	<0.012
Among sites within watersheds	11	49.21	0.340	27.6%	0.558	<0.001
Within sites	210	114.58	0.546	44.2%	0.384	<0.001
Total	227	262.63	1.234			

Table S6. ddRAD SNP pairwise F_{ST} (Wright 1951; Weir and Cockerham 1984) below the diagonal, and *p*-values based on 1,000 bootstrap resamplings above the diagonal from 55,267 SNPs for samples grouped by **(A)** sampling site, **(B)** watershed, and **(c)** as implemented in the R package StAMPP (Pembleton et al., 2013).

(A)	**01-DUK**	**02-ORB**	**03-NGO**	**04-BOL**	**05-CHU**	**06-ACA**	**07-APU**	**08-OCA**	**09-UWA**	**10-OCU**	**11-OT**	**12-BN**	**13-MF**	**14-MS**	**15-KAF**	**16- JN**	**17-NB**	**18-WAM**
01-DUK		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
02-ORB	0.043		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
03-NGO	0.056	0.046		<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
04-BOL	0.067	0.062	0.020		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
05-CHU	0.100	0.076	0.027	0.029		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
06-ACA	0.052	0.058	0.009	0.026	0.035		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
07-APU	0.036	0.046	-0.012	0.024	0.036	0.014		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
08-OCA	0.079	0.070	0.029	0.030	0.055	0.034	0.042		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
09-UWA	0.056	0.041	0.005	0.022	0.037	0.018	0.018	0.004		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
10-OCU	0.097	0.093	0.042	0.016	0.035	0.052	0.038	0.066	0.054		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
11-OT	0.101	0.096	0.043	0.037	0.047	0.058	0.047	0.084	0.063	0.015		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12-BN	0.133	0.126	0.103	0.102	0.107	0.110	0.101	0.133	0.085	0.104	0.109		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
13-MF	0.284	0.295	0.308	0.325	0.355	0.315	0.306	0.323	0.241	0.360	0.368	0.262		<0.001	<0.001	<0.001	<0.001	<0.001
14-MS	0.271	0.281	0.295	0.316	0.346	0.305	0.293	0.311	0.232	0.351	0.353	0.265	0.075		<0.001	<0.001	<0.001	<0.001
15-KAF	0.277	0.295	0.305	0.322	0.352	0.311	0.301	0.319	0.244	0.354	0.357	0.270	0.077	0.112		<0.001	<0.001	<0.001
16-JN	0.369	0.384	0.395	0.411	0.449	0.402	0.403	0.409	0.325	0.446	0.462	0.333	0.119	0.182	0.137		<0.001	<0.001
17-NB	0.382	0.397	0.409	0.422	0.461	0.414	0.417	0.420	0.340	0.457	0.472	0.334	0.159	0.201	0.186	0.150		<0.001
18-WAM	0.336	0.345	0.356	0.361	0.404	0.362	0.365	0.348	0.281	0.397	0.415	0.302	0.189	0.208	0.184	0.189	0.144	

(B)	**Albert Nile**	**Achwa River**	**Okole River**	**Lake Kyoga**	**Lake Albert**	**Kafu River**	**Lake Victoria**
Albert Nile		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Achwa River	0.051		<0.001	<0.001	<0.001	<0.001	<0.001
Okole River	0.040	0.010		<0.001	<0.001	<0.001	<0.001
Lake Kyoga	0.076	0.022	0.043		<0.001	<0.001	<0.001
Lake Albert	0.259	0.305	0.272	0.303		<0.001	<0.001
Kafu River	0.273	0.321	0.286	0.320	0.078		<0.001
Lake Victoria	0.330	0.364	0.328	0.357	0.132	0.151	

(C)

	NW	**“b”**	**“a”**	**NE**	**W**	**“c”**	**“d”**	**S**
Northwest		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zone “b”	0.025		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zone “a”	0.024	0.011		<0.001	<0.001	<0.001	<0.001	<0.001
Northeast	0.060	0.030	0.060		<0.001	<0.001	<0.001	<0.001
West	0.136	0.147	0.106	0.164		<0.001	<0.001	<0.001
“c”	0.259	0.296	0.227	0.341	0.152		<0.001	<0.001
“d”	0.279	0.316	0.251	0.361	0.167	0.066		<0.001
South	0.313	0.339	0.281	0.397	0.179	0.171	0.158	

Table S7. ddRAD SNP and microsatellite (µsat) *p*-values corrected for multiple testing of pairwise F_{ST} (Wright 1951; Weir and Cockerham 1984), and geographic distance in km (Geo. dist.) used in isolation by distance analysis for the estimates made by **(a)** population, **(b)** watershed, and **(c)** by the four genetic units and the admixture zones. For the 55,267 ddRAD SNPs (RAD), *p*-values are based on bootstrap (*n* = 1,000) sampling in the R package "StAMPP" (Pembleton et al., 2013). For the 16 microsatellites, *p*-values are based on 1000 randomizations in FSTAT. For corrections, we used Benjamini-Hochberg (BH) method (Benjamini and Hochberg, 1995) and the Bonferroni (BF) method (Dunn 1961) in the R package "stats" (R Core Team, 2016).

(a)

Sample 1	Sample 2	Geo. dist.	ddRAD F_{ST}	ddRAD *p*-value	ddRAD BH	ddRAD BF	µsat F_{ST}	µsat *p*-value	µsat BH	µsat BF
01-DUK	02-ORB	86.9	0.043	0.0000	0.0000	0.0006	0.002	0.0137	0.0147	1.0000
01-DUK	03-NGO	168.7	0.056	0.0000	0.0000	0.0006	0.013	0.0003	0.0005	0.0505
01-DUK	04-BOL	185.0	0.067	0.0000	0.0000	0.0006	0.033	0.0003	0.0005	0.0505
01-DUK	05-CHU	213.8	0.100	0.0000	0.0000	0.0006	0.032	0.0007	0.0009	0.0995
01-DUK	06-ACA	190.0	0.052	0.0000	0.0000	0.0006	0.029	0.0003	0.0005	0.0505
01-DUK	07-APU	216.5	0.036	0.0000	0.0000	0.0006	0.046	0.0003	0.0005	0.0505
01-DUK	08-OCA	190.8	0.079	0.0000	0.0000	0.0006	0.057	0.0003	0.0005	0.0505
01-DUK	09-UWA	123.5	0.056	0.0000	0.0000	0.0006	0.137	0.0003	0.0005	0.0505
01-DUK	10-OCU	274.6	0.097	0.0000	0.0000	0.0006	0.076	0.0003	0.0005	0.0505
01-DUK	11-OT	282.2	0.101	0.0000	0.0000	0.0006	0.059	0.0003	0.0005	0.0505
01-DUK	12-BN	326.6	0.133	0.0000	0.0000	0.0006	0.119	0.0007	0.0009	0.0995
01-DUK	13-MF	167.9	0.284	0.0000	0.0000	0.0006	0.094	0.0003	0.0005	0.0505
01-DUK	14-MS	191.9	0.271	0.0000	0.0000	0.0006	0.151	0.0003	0.0005	0.0505
01-DUK	15-KAF	216.7	0.277	0.0000	0.0000	0.0006	0.150	0.0003	0.0005	0.0505
01-DUK	16-JN	278.1	0.369	0.0000	0.0000	0.0006	0.149	0.0010	0.0012	0.1499
01-DUK	17-NB	440.9	0.382	0.0000	0.0000	0.0006	0.141	0.0003	0.0005	0.0505
01-DUK	18-WAM	405.4	0.336	0.0000	0.0000	0.0006	0.187	0.0003	0.0005	0.0505
02-ORB	03-NGO	90.1	0.046	0.0000	0.0000	0.0006	0.022	0.0003	0.0005	0.0505
02-ORB	04-BOL	121.3	0.062	0.0000	0.0000	0.0006	0.055	0.0003	0.0005	0.0505
02-ORB	05-CHU	176.5	0.076	0.0000	0.0000	0.0006	0.035	0.0003	0.0005	0.0505
02-ORB	06-ACA	178.5	0.058	0.0000	0.0000	0.0006	0.043	0.0003	0.0005	0.0505
02-ORB	07-APU	205.3	0.046	0.0000	0.0000	0.0006	0.057	0.0003	0.0005	0.0505
02-ORB	08-OCA	169.5	0.070	0.0000	0.0000	0.0006	0.073	0.0003	0.0005	0.0505
02-ORB	09-UWA	158.9	0.041	0.0000	0.0000	0.0006	0.175	0.0003	0.0005	0.0505
02-ORB	10-OCU	255.9	0.093	0.0000	0.0000	0.0006	0.094	0.0003	0.0005	0.0505
02-ORB	11-OT	260.3	0.096	0.0000	0.0000	0.0006	0.066	0.0003	0.0005	0.0505
02-ORB	12-BN	307.6	0.126	0.0000	0.0000	0.0006	0.144	0.0007	0.0009	0.0995
02-ORB	13-MF	169.0	0.295	0.0000	0.0000	0.0006	0.141	0.0003	0.0005	0.0505
02-ORB	14-MS	229.8	0.281	0.0000	0.0000	0.0006	0.194	0.0003	0.0005	0.0505
02-ORB	15-KAF	241.1	0.295	0.0000	0.0000	0.0006	0.182	0.0003	0.0005	0.0505
02-ORB	16-JN	282.4	0.384	0.0000	0.0000	0.0006	0.188	0.0010	0.0012	0.1499
02-ORB	17-NB	428.2	0.397	0.0000	0.0000	0.0006	0.184	0.0003	0.0005	0.0505
02-ORB	18-WAM	408.9	0.345	0.0000	0.0000	0.0006	0.240	0.0003	0.0005	0.0505
03-NGO	04-BOL	48.1	0.020	0.0000	0.0000	0.0006	0.028	0.0003	0.0005	0.0505
03-NGO	05-CHU	124.4	0.027	0.0000	0.0000	0.0006	0.039	0.0003	0.0005	0.0505
03-NGO	06-ACA	155.9	0.009	0.0000	0.0000	0.0006	0.059	0.0003	0.0005	0.0505
03-NGO	07-APU	177.3	-0.012	1.0000	1.0000	1.0000	0.055	0.0003	0.0005	0.0505

03-NGO	08-OCA	138.3	0.029	0.0000	0.0000	0.0006	0.082	0.0003	0.0005	0.0505
03-NGO	09-UWA	188.3	0.005	0.0000	0.0000	0.0006	0.185	0.0003	0.0005	0.0505
03-NGO	10-OCU	212.2	0.042	0.0000	0.0000	0.0006	0.096	0.0003	0.0005	0.0505
03-NGO	11-OT	212.8	0.043	0.0000	0.0000	0.0006	0.082	0.0003	0.0005	0.0505
03-NGO	12-BN	259.1	0.103	0.0000	0.0000	0.0006	0.123	0.0003	0.0005	0.0505
03-NGO	13-MF	162.7	0.308	0.0000	0.0000	0.0006	0.136	0.0003	0.0005	0.0505
03-NGO	14-MS	248.1	0.295	0.0000	0.0000	0.0006	0.205	0.0007	0.0009	0.0995
03-NGO	15-KAF	244.3	0.305	0.0000	0.0000	0.0006	0.189	0.0003	0.0005	0.0505
03-NGO	16-JN	260.0	0.395	0.0000	0.0000	0.0006	0.192	0.0013	0.0016	0.2004
03-NGO	17-NB	379.5	0.409	0.0000	0.0000	0.0006	0.214	0.0003	0.0005	0.0505
03-NGO	18-WAM	376.9	0.356	0.0000	0.0000	0.0006	0.212	0.0003	0.0005	0.0505
04-BOL	05-CHU	77.4	0.029	0.0000	0.0000	0.0006	0.013	0.0150	0.0160	1.0000
04-BOL	06-ACA	117.3	0.026	0.0000	0.0000	0.0006	0.021	0.0003	0.0005	0.0505
04-BOL	07-APU	135.2	0.024	0.0000	0.0000	0.0006	0.010	0.0020	0.0023	0.2999
04-BOL	08-OCA	97.5	0.030	0.0000	0.0000	0.0006	0.035	0.0003	0.0005	0.0505
04-BOL	09-UWA	171.3	0.022	0.0000	0.0000	0.0006	0.195	0.0003	0.0005	0.0505
04-BOL	10-OCU	165.3	0.016	0.0000	0.0000	0.0006	0.060	0.0003	0.0005	0.0505
04-BOL	11-OT	165.2	0.037	0.0000	0.0000	0.0006	0.042	0.0003	0.0005	0.0505
04-BOL	12-BN	211.2	0.102	0.0000	0.0000	0.0006	0.122	0.0003	0.0005	0.0505
04-BOL	13-MF	130.6	0.325	0.0000	0.0000	0.0006	0.156	0.0003	0.0005	0.0505
04-BOL	14-MS	221.5	0.316	0.0000	0.0000	0.0006	0.228	0.0003	0.0005	0.0505
04-BOL	15-KAF	211.2	0.322	0.0000	0.0000	0.0006	0.193	0.0003	0.0005	0.0505
04-BOL	16-JN	217.3	0.411	0.0000	0.0000	0.0006	0.191	0.0003	0.0005	0.0505
04-BOL	17-NB	331.5	0.422	0.0000	0.0000	0.0006	0.225	0.0003	0.0005	0.0505
04-BOL	18-WAM	330.7	0.361	0.0000	0.0000	0.0006	0.217	0.0003	0.0005	0.0505
05-CHU	06-ACA	59.7	0.035	0.0000	0.0000	0.0006	0.016	0.0598	0.0614	1.0000
05-CHU	07-APU	65.6	0.036	0.0000	0.0000	0.0006	0.004	0.4382	0.4382	1.0000
05-CHU	08-OCA	39.8	0.055	0.0000	0.0000	0.0006	0.043	0.0023	0.0026	0.3504
05-CHU	09-UWA	149.5	0.037	0.0000	0.0000	0.0006	0.192	0.0003	0.0005	0.0505
05-CHU	10-OCU	87.9	0.035	0.0000	0.0000	0.0006	0.049	0.0003	0.0005	0.0505
05-CHU	11-OT	88.6	0.047	0.0000	0.0000	0.0006	0.015	0.0186	0.0195	1.0000
05-CHU	12-BN	135.6	0.107	0.0000	0.0000	0.0006	0.123	0.0007	0.0009	0.0995
05-CHU	13-MF	86.4	0.355	0.0000	0.0000	0.0006	0.156	0.0003	0.0005	0.0505
05-CHU	14-MS	176.3	0.346	0.0000	0.0000	0.0006	0.225	0.0007	0.0009	0.0995
05-CHU	15-KAF	154.5	0.352	0.0000	0.0000	0.0006	0.166	0.0003	0.0005	0.0505
05-CHU	16-JN	143.4	0.449	0.0000	0.0000	0.0006	0.190	0.0007	0.0009	0.0995
05-CHU	17-NB	256.7	0.461	0.0000	0.0000	0.0006	0.201	0.0003	0.0005	0.0505
05-CHU	18-WAM	253.3	0.404	0.0000	0.0000	0.0006	0.224	0.0003	0.0005	0.0505
06-ACA	07-APU	27.4	0.014	0.0000	0.0000	0.0006	0.017	0.0196	0.0204	1.0000
06-ACA	08-OCA	21.2	0.034	0.0000	0.0000	0.0006	0.003	0.0690	0.0703	1.0000
06-ACA	09-UWA	98.4	0.018	0.0000	0.0000	0.0006	0.174	0.0003	0.0005	0.0505
06-ACA	10-OCU	84.6	0.052	0.0000	0.0000	0.0006	0.052	0.0003	0.0005	0.0505
06-ACA	11-OT	92.7	0.058	0.0000	0.0000	0.0006	0.049	0.0003	0.0005	0.0505
06-ACA	12-BN	136.6	0.110	0.0000	0.0000	0.0006	0.091	0.0003	0.0005	0.0505
06-ACA	13-MF	30.4	0.315	0.0000	0.0000	0.0006	0.129	0.0003	0.0005	0.0505
06-ACA	14-MS	116.6	0.305	0.0000	0.0000	0.0006	0.206	0.0010	0.0012	0.1499
06-ACA	15-KAF	96.6	0.311	0.0000	0.0000	0.0006	0.174	0.0003	0.0005	0.0505
06-ACA	16-JN	106.3	0.402	0.0000	0.0000	0.0006	0.185	0.0010	0.0012	0.1499
06-ACA	17-NB	253.4	0.414	0.0000	0.0000	0.0006	0.189	0.0003	0.0005	0.0505

06-ACA	18-WAM	230.7	0.362	0.0000	0.0000	0.0006	0.207	0.0003	0.0005	0.0505
07-APU	08-OCA	39.1	0.042	0.0000	0.0000	0.0006	0.036	0.0003	0.0005	0.0505
07-APU	09-UWA	117.7	0.018	0.0000	0.0000	0.0006	0.203	0.0003	0.0005	0.0505
07-APU	10-OCU	59.0	0.038	0.0000	0.0000	0.0006	0.058	0.0003	0.0005	0.0505
07-APU	11-OT	68.3	0.047	0.0000	0.0000	0.0006	0.037	0.0007	0.0009	0.0995
07-APU	12-BN	110.2	0.101	0.0000	0.0000	0.0006	0.141	0.0007	0.0009	0.0995
07-APU	13-MF	51.1	0.306	0.0000	0.0000	0.0006	0.156	0.0003	0.0005	0.0505
07-APU	14-MS	120.5	0.293	0.0000	0.0000	0.0006	0.244	0.0007	0.0009	0.0995
07-APU	15-KAF	92.0	0.301	0.0000	0.0000	0.0006	0.211	0.0003	0.0005	0.0505
07-APU	16-JN	82.8	0.403	0.0000	0.0000	0.0006	0.220	0.0023	0.0026	0.3504
07-APU	17-NB	226.0	0.417	0.0000	0.0000	0.0006	0.244	0.0010	0.0012	0.1499
07-APU	18-WAM	204.5	0.365	0.0000	0.0000	0.0006	0.227	0.0003	0.0005	0.0505
08-OCA	09-UWA	111.8	0.004	0.0000	0.0000	0.0006	0.194	0.0003	0.0005	0.0505
08-OCA	10-OCU	87.0	0.066	0.0000	0.0000	0.0006	0.046	0.0003	0.0005	0.0505
08-OCA	11-OT	92.8	0.084	0.0000	0.0000	0.0006	0.075	0.0003	0.0005	0.0505
08-OCA	12-BN	139.3	0.133	0.0000	0.0000	0.0006	0.117	0.0003	0.0005	0.0505
08-OCA	13-MF	46.7	0.323	0.0000	0.0000	0.0006	0.149	0.0003	0.0005	0.0505
08-OCA	14-MS	137.0	0.311	0.0000	0.0000	0.0006	0.230	0.0003	0.0005	0.0505
08-OCA	15-KAF	117.8	0.319	0.0000	0.0000	0.0006	0.195	0.0003	0.0005	0.0505
08-OCA	16-JN	121.8	0.409	0.0000	0.0000	0.0006	0.206	0.0013	0.0016	0.2004
08-OCA	17-NB	258.9	0.420	0.0000	0.0000	0.0006	0.218	0.0003	0.0005	0.0505
08-OCA	18-WAM	242.6	0.348	0.0000	0.0000	0.0006	0.236	0.0003	0.0005	0.0505
09-UWA	10-OCU	175.3	0.054	0.0000	0.0000	0.0006	0.210	0.0003	0.0005	0.0505
09-UWA	11-OT	185.5	0.063	0.0000	0.0000	0.0006	0.225	0.0003	0.0005	0.0505
09-UWA	12-BN	223.1	0.085	0.0000	0.0000	0.0006	0.179	0.0003	0.0005	0.0505
09-UWA	13-MF	68.2	0.241	0.0000	0.0000	0.0006	0.034	0.0046	0.0050	0.7007
09-UWA	14-MS	71.7	0.232	0.0000	0.0000	0.0006	0.121	0.0003	0.0005	0.0505
09-UWA	15-KAF	93.2	0.244	0.0000	0.0000	0.0006	0.129	0.0003	0.0005	0.0505
09-UWA	16-JN	159.7	0.325	0.0000	0.0000	0.0006	0.119	0.0007	0.0009	0.0995
09-UWA	17-NB	328.1	0.340	0.0000	0.0000	0.0006	0.081	0.0003	0.0005	0.0505
09-UWA	18-WAM	284.8	0.281	0.0000	0.0000	0.0006	0.159	0.0003	0.0005	0.0505
10-OCU	11-OT	11.9	0.015	0.0000	0.0000	0.0006	0.019	0.1281	0.1298	1.0000
10-OCU	12-BN	52.4	0.104	0.0000	0.0000	0.0006	0.123	0.0007	0.0009	0.0995
10-OCU	13-MF	110.0	0.360	0.0000	0.0000	0.0006	0.169	0.0003	0.0005	0.0505
10-OCU	14-MS	164.5	0.351	0.0000	0.0000	0.0006	0.224	0.0003	0.0005	0.0505
10-OCU	15-KAF	127.6	0.354	0.0000	0.0000	0.0006	0.198	0.0003	0.0005	0.0505
10-OCU	16-JN	74.1	0.446	0.0000	0.0000	0.0006	0.242	0.0007	0.0009	0.0995
10-OCU	17-NB	172.3	0.457	0.0000	0.0000	0.0006	0.268	0.0003	0.0005	0.0505
10-OCU	18-WAM	166.0	0.397	0.0000	0.0000	0.0006	0.253	0.0003	0.0005	0.0505
11-OT	12-BN	47.5	0.109	0.0000	0.0000	0.0006	0.143	0.0010	0.0012	0.1499
11-OT	13-MF	119.4	0.368	0.0000	0.0000	0.0006	0.192	0.0003	0.0005	0.0505
11-OT	14-MS	176.3	0.353	0.0000	0.0000	0.0006	0.250	0.0007	0.0009	0.0995
11-OT	15-KAF	139.6	0.357	0.0000	0.0000	0.0006	0.188	0.0003	0.0005	0.0505
11-OT	16-JN	84.3	0.462	0.0000	0.0000	0.0006	0.237	0.0020	0.0023	0.2999
11-OT	17-NB	168.7	0.472	0.0000	0.0000	0.0006	0.251	0.0007	0.0009	0.0995
11-OT	18-WAM	168.2	0.415	0.0000	0.0000	0.0006	0.261	0.0003	0.0005	0.0505
12-BN	13-MF	160.5	0.262	0.0000	0.0000	0.0006	0.150	0.0010	0.0012	0.1499
12-BN	14-MS	201.3	0.265	0.0000	0.0000	0.0006	0.212	0.0056	0.0060	0.8507
12-BN	15-KAF	161.4	0.270	0.0000	0.0000	0.0006	0.220	0.0007	0.0009	0.0995

12-BN	16-JN	88.4	0.333	0.0000	0.0000	0.0006	0.216	0.0062	0.0067	0.9501
12-BN	17-NB	121.2	0.334	0.0000	0.0000	0.0006	0.183	0.0020	0.0023	0.2999
12-BN	18-WAM	129.8	0.302	0.0000	0.0000	0.0006	0.221	0.0007	0.0009	0.0995
13-MF	14-MS	92.7	0.075	0.0000	0.0000	0.0006	0.096	0.0010	0.0012	0.1499
13-MF	15-KAF	81.6	0.077	0.0000	0.0000	0.0006	0.113	0.0010	0.0012	0.1499
13-MF	16-JN	114.4	0.119	0.0000	0.0000	0.0006	0.084	0.0180	0.0190	1.0000
13-MF	17-NB	273.0	0.159	0.0000	0.0000	0.0006	0.084	0.0016	0.0019	0.2494
13-MF	18-WAM	242.2	0.189	0.0000	0.0000	0.0006	0.139	0.0003	0.0005	0.0505
14-MS	15-KAF	40.8	0.112	0.0000	0.0000	0.0006	0.146	0.0003	0.0005	0.0505
14-MS	16-JN	119.6	0.182	0.0000	0.0000	0.0006	0.239	0.0046	0.0050	0.7007
14-MS	17-NB	287.8	0.201	0.0000	0.0000	0.0006	0.235	0.0033	0.0036	0.5003
14-MS	18-WAM	231.6	0.208	0.0000	0.0000	0.0006	0.289	0.0013	0.0016	0.2004
15-KAF	16-JN	78.9	0.137	0.0000	0.0000	0.0006	0.149	0.0033	0.0036	0.5003
15-KAF	17-NB	247.7	0.186	0.0000	0.0000	0.0006	0.213	0.0007	0.0009	0.0995
15-KAF	18-WAM	194.9	0.184	0.0000	0.0000	0.0006	0.238	0.0003	0.0005	0.0505
16-JN	17-NB	170.1	0.150	0.0000	0.0000	0.0006	0.071	0.0369	0.0382	1.0000
16-JN	18-WAM	128.0	0.189	0.0000	0.0000	0.0006	0.096	0.0029	0.0033	0.4498
17-NB	18-WAM	83.2	0.144	0.0000	0.0000	0.0006	-0.021	0.3931	0.3957	1.0000

(b) Sample 1	Sample 2	ddRAD F_{ST}	ddRAD *p*-value	ddRAD BH	ddRAD BF	µsat F_{ST}	µsat *p*-value	µsat BH	µsat BF
Albert Nile	Achwa River	0.025	0.0000	0.0000	0.0001	0.025	0.00238	0.00238	0.04998
Albert Nile	Okole River	0.035	0.0000	0.0000	0.0001	0.035	0.00238	0.00238	0.04998
Albert Nile	Lake Kyoga	0.063	0.0000	0.0000	0.0001	0.063	0.00238	0.00238	0.04998
Albert Nile	Lake Albert	0.119	0.0000	0.0000	0.0001	0.119	0.00238	0.00238	0.04998
Albert Nile	Kafu River	0.155	0.0000	0.0000	0.0001	0.155	0.00238	0.00238	0.04998
Albert Nile	Lake Victoria	0.186	0.0000	0.0000	0.0001	0.186	0.00238	0.00238	0.04998
Achwa River	Okole River	0.029	0.0000	0.0000	0.0001	0.029	0.00238	0.00238	0.04998
Achwa River	Lake Kyoga	0.040	0.0000	0.0000	0.0001	0.040	0.00238	0.00238	0.04998
Achwa River	Lake Albert	0.158	0.0000	0.0000	0.0001	0.158	0.00238	0.00238	0.04998
Achwa River	Kafu River	0.170	0.0000	0.0000	0.0001	0.170	0.00238	0.00238	0.04998
Achwa River	Lake Victoria	0.190	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Okole River	Lake Kyoga	0.037	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
Okole River	Lake Albert	0.095	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
Okole River	Kafu River	0.124	0.0000	0.0000	0.0001	0.124	0.00238	0.00238	0.04998
Okole River	Lake Victoria	0.145	0.0000	0.0000	0.0001	0.145	0.00238	0.00238	0.04998
Lake Kyoga	Lake Albert	0.155	0.0000	0.0000	0.0001	0.155	0.00238	0.00238	0.04998
Lake Kyoga	Kafu River	0.168	0.0000	0.0000	0.0001	0.168	0.00238	0.00238	0.04998
Lake Kyoga	Lake Victoria	0.205	0.0000	0.0000	0.0001	0.205	0.00238	0.00238	0.04998
Lake Albert	Kafu River	0.098	0.0000	0.0000	0.0001	0.098	0.00238	0.00238	0.04998
Lake Albert	Lake Victoria	0.159	0.0000	0.0000	0.0001	0.159	0.00238	0.00238	0.04998
Kafu River	Lake Victoria	0.208	0.0000	0.0000	0.0001	0.208	0.00238	0.00238	0.04998

(c) Sample 1	Sample 2	ddRAD F_{ST}	ddRAD *p*-value	ddRAD BH	ddRAD BF	µsat F_{ST}	µsat *p*-value	µsat BH	µsat BF
Northwest	Zone “b”	0.025	0.0000	0.0000	0.0001	0.025	0.00238	0.00238	0.04998
Northwest	Zone “a”	0.024	0.0000	0.0000	0.0001	0.035	0.00238	0.00238	0.04998
Northwest	Northeast	0.060	0.0000	0.0000	0.0001	0.063	0.00238	0.00238	0.04998
Northwest	West	0.136	0.0000	0.0000	0.0001	0.119	0.00238	0.00238	0.04998
Northwest	Zone “c”	0.259	0.0000	0.0000	0.0001	0.155	0.00238	0.00238	0.04998
Northwest	Zone “d”	0.279	0.0000	0.0000	0.0001	0.186	0.00238	0.00238	0.04998

Northwest	South	0.313	0.0000	0.0000	0.0001	0.029	0.00238	0.00238	0.04998
Zone “b”	Zone “a”	0.011	0.0000	0.0000	0.0001	0.158	0.00238	0.00238	0.04998
Zone “b”	Northeast	0.030	0.0000	0.0000	0.0001	0.170	0.00238	0.00238	0.04998
Zone “b”	West	0.147	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Zone “b”	Zone “c”	0.296	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Zone “b”	Zone “d”	0.316	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
Zone “b”	South	0.339	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
Zone “a”	Northeast	0.060	0.0000	0.0000	0.0001	0.170	0.00238	0.00238	0.04998
Zone “a”	West	0.106	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Zone “a”	Zone “c”	0.227	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Zone “a”	Zone “d”	0.251	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
Zone “a”	South	0.281	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
Northeast	West	0.164	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Northeast	Zone “c”	0.341	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
Northeast	Zone “d”	0.361	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
Northeast	South	0.397	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
West	Zone “c”	0.152	0.0000	0.0000	0.0001	0.190	0.00238	0.00238	0.04998
West	Zone “d”	0.167	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
West	South	0.179	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
Zone “c”	Zone “d”	0.066	0.0000	0.0000	0.0001	0.037	0.00238	0.00238	0.04998
Zone “c”	South	0.171	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998
Zone “d”	South	0.158	0.0000	0.0000	0.0001	0.095	0.00238	0.00238	0.04998

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Table S8. Individual fly genotypes and haplotypes. Microsatellite cluster analysis assignments from STRUCTURE (Figure S5; Pritchard et al., 2000; Falush et al., 2003), mtDNA haplotype and haplogroup assignments (Figure S1), and ddRAD SNP cluster analysis assignment (q-value) from STRUCTURE (Figure S5), FINESTRUCTURE cluster (FC; Figure 6), and watershed of origin.

Site Code	Fly No.	Q-value 1 (K=2) for µsat	Q-value 2 (K=2) for µsat	Haplotype assignment	Haplogroup assignment	Q-value 1 (K=2) for ddRAD	Q-value 2 (K=2) for ddRAD	FC	Watershed
01-DUK	1	0.922	0.078	A2	A	0.944	0.056	3	Albert Nile
01-DUK	2	0.982	0.018	A2	A	0.950	0.050	3	Albert Nile
01-DUK	3	0.596	0.405	A2	A	0.958	0.042	2	Albert Nile
01-DUK	4	0.963	0.037	A2	A	0.935	0.065	2	Albert Nile
01-DUK	5	0.988	0.012	A2	A	0.944	0.056	2	Albert Nile
01-DUK	6	0.795	0.205	A12	A	0.948	0.052	3	Albert Nile
01-DUK	7	0.904	0.096	A2	A	0.854	0.146	3	Albert Nile
01-DUK	8	0.993	0.007	A3	A	0.946	0.054	2	Albert Nile
01-DUK	9	0.982	0.018	A6	A	0.924	0.076	3	Albert Nile
01-DUK	10	0.955	0.045	A2	A	0.614	0.386	7	Albert Nile
01-DUK	11	0.334	0.666	A2	A	0.848	0.152	3	Albert Nile
01-DUK	12	0.965	0.035	A2	A	0.952	0.048	2	Albert Nile
01-DUK	13	0.987	0.013	A2	A	0.932	0.068	2	Albert Nile
01-DUK	14	0.929	0.071	A1	A	0.948	0.052	3	Albert Nile
01-DUK	15	0.963	0.037	A2	A	0.945	0.055	2	Albert Nile
01-DUK	16	0.962	0.038	A2	A	0.937	0.063	2	Albert Nile
01-DUK	17	0.942	0.058	A2	A	0.919	0.081	3	Albert Nile
01-DUK	18	0.993	0.007	A2	A	0.972	0.028	3	Albert Nile
01-DUK	19	0.935	0.065	A2	A	0.941	0.059	2	Albert Nile
01-DUK	20	0.985	0.015	A2	A	0.975	0.025	3	Albert Nile
02-ORB	21	0.837	0.163	A2	A	0.922	0.078	2	Albert Nile
02-ORB	22	0.985	0.015	A2	A	0.909	0.091	2	Albert Nile
02-ORB	23	0.994	0.006	B1	B	0.926	0.074	3	Albert Nile
02-ORB	24	0.967	0.033	A2	A	0.922	0.078	2	Albert Nile
02-ORB	25	0.982	0.018	A2	A	0.930	0.070	3	Albert Nile
02-ORB	26	0.964	0.036	B1	B	0.908	0.092	2	Albert Nile
02-ORB	27	0.971	0.030	A1	A	0.920	0.080	3	Albert Nile
02-ORB	28	0.840	0.160	A2	A	0.886	0.114	3	Albert Nile
02-ORB	29	0.956	0.044	A1	A	0.935	0.065	3	Albert Nile
02-ORB	30	0.996	0.004	A2	A	0.930	0.070	3	Albert Nile
02-ORB	31	0.863	0.137	A2	A	0.859	0.141	3	Albert Nile
02-ORB	32	0.962	0.038	A2	A	0.884	0.116	3	Albert Nile
03-NGO	33	0.988	0.012	B3	B	0.970	0.030	6	Achwa River
03-NGO	34	0.991	0.009	B1	B	1.000	0.000	4	Achwa River
03-NGO	35	0.938	0.063	A6	A	1.000	0.000	4	Achwa River
03-NGO	36	0.977	0.023	B1	B	1.000	0.000	4	Achwa River
03-NGO	37	0.910	0.090	B1	B	0.881	0.119	6	Achwa River
03-NGO	38	0.951	0.049	B1	B	1.000	0.000	4	Achwa River
03-NGO	39	0.996	0.004	B1	B	0.874	0.126	3	Achwa River
03-NGO	40	0.725	0.276	B1	B	1.000	0.000	4	Achwa River
03-NGO	41	0.965	0.035	A6	A	0.999	0.001	2	Achwa River
03-NGO	42	0.963	0.037	B1	B	1.000	0.000	2	Achwa River

03-NGO	43	0.788	0.212	B1	B	1.000	0.000	4	Achwa River
03-NGO	44	0.992	0.008	B1	B	1.000	0.000	1	Achwa River
04-BOL	45	0.992	0.008	A10	A	1.000	0.000	4	Achwa River
04-BOL	46	0.934	0.066	B1	B	1.000	0.000	4	Achwa River
04-BOL	47	0.991	0.009	N/A	N/A	1.000	0.000	4	Achwa River
04-BOL	48	0.994	0.006	N/A	N/A	1.000	0.000	4	Achwa River
04-BOL	49	0.962	0.038	B1	B	1.000	0.000	4	Achwa River
04-BOL	50	0.950	0.051	B1	B	1.000	0.000	4	Achwa River
04-BOL	51	0.994	0.006	B1	B	1.000	0.000	4	Achwa River
04-BOL	52	0.994	0.006	A2	A	1.000	0.000	1	Achwa River
04-BOL	53	0.991	0.009	A2	A	1.000	0.000	4	Achwa River
04-BOL	54	0.872	0.128	B1	B	0.982	0.018	4	Achwa River
04-BOL	55	0.989	0.011	B1	B	1.000	0.000	5	Achwa River
04-BOL	56	0.978	0.023	N/A	N/A	0.971	0.029	6	Achwa River
04-BOL	57	0.980	0.020	N/A	N/A	0.986	0.014	1	Achwa River
04-BOL	58	0.994	0.006	B1	B	1.000	0.000	1	Achwa River
04-BOL	59	0.986	0.014	B1	B	1.000	0.000	4	Achwa River
04-BOL	60	0.979	0.021	N/A	N/A	1.000	0.000	1	Achwa River
04-BOL	61	0.927	0.073	N/A	N/A	0.982	0.018	4	Achwa River
04-BOL	62	0.995	0.005	B1	B	0.877	0.123	6	Achwa River
04-BOL	63	0.972	0.028	A2	A	1.000	0.000	5	Achwa River
04-BOL	64	0.996	0.004	B1	B	0.985	0.015	6	Achwa River
05-CHU	65	0.983	0.017	A2	A	1.000	0.000	4	Achwa River
05-CHU	66	0.994	0.006	A2	A	0.999	0.001	4	Achwa River
05-CHU	67	0.981	0.019	B1	B	1.000	0.000	4	Achwa River
05-CHU	68	0.970	0.030	B6	B	0.999	0.001	6	Achwa River
05-CHU	69	0.979	0.021	B1	B	1.000	0.000	4	Achwa River
05-CHU	70	0.994	0.006	A2	A	1.000	0.000	4	Achwa River
05-CHU	71	0.986	0.014	A2	A	0.989	0.011	4	Achwa River
05-CHU	72	0.934	0.066	B1	B	1.000	0.000	4	Achwa River
05-CHU	73	0.992	0.008	A2	A	0.998	0.002	4	Achwa River
05-CHU	74	0.995	0.005	A2	A	1.000	0.000	4	Achwa River
05-CHU	75	0.937	0.063	B17	B	1.000	0.000	4	Achwa River
05-CHU	76	0.985	0.015	B19	B	0.999	0.001	4	Achwa River
05-CHU	77	0.986	0.014	B1	B	1.000	0.000	4	Achwa River
06-ACA	78	0.981	0.019	B1	B	1.000	0.000	3	Okole River
06-ACA	79	0.971	0.029	B1	B	1.000	0.000	4	Okole River
06-ACA	80	0.950	0.050	B1	B	1.000	0.000	4	Okole River
06-ACA	81	0.991	0.009	B2	B	0.951	0.049	3	Okole River
06-ACA	82	0.989	0.011	B1	B	1.000	0.000	1	Okole River
06-ACA	83	0.964	0.036	A1	A	1.000	0.000	3	Okole River
06-ACA	84	0.994	0.006	B1	B	1.000	0.000	3	Okole River
06-ACA	85	0.987	0.013	B1	B	1.000	0.000	3	Okole River
06-ACA	86	0.991	0.009	B2	B	0.947	0.053	3	Okole River
06-ACA	87	0.989	0.011	B2	B	0.826	0.174	1	Okole River
06-ACA	88	0.974	0.026	N/A	N/A	0.987	0.013	4	Okole River
06-ACA	89	0.994	0.006	B1	B	0.995	0.005	1	Okole River
06-ACA	90	0.975	0.026	A1	A	0.998	0.002	4	Okole River
06-ACA	91	0.992	0.008	B1	B	1.000	0.000	4	Okole River

06-ACA	92	0.993	0.008	B18	B	0.987	0.013	4	Okole River
06-ACA	93	0.965	0.035	A2	A	0.967	0.033	6	Okole River
06-ACA	94	0.953	0.047	B6	B	0.923	0.077	6	Okole River
06-ACA	95	0.953	0.047	A1	A	0.973	0.027	1	Okole River
07-APU	96	0.995	0.006	B1	B	0.957	0.043	3	Okole River
07-APU	97	0.994	0.006	B2	B	1.000	0.000	1	Okole River
07-APU	98	0.973	0.027	B1	B	1.000	0.000	4	Okole River
07-APU	99	0.993	0.007	B1	B	0.931	0.069	3	Okole River
07-APU	100	0.940	0.060	B1	B	0.926	0.074	3	Okole River
07-APU	101	0.989	0.011	B1	B	1.000	0.000	1	Okole River
07-APU	102	0.955	0.045	B2	B	1.000	0.000	5	Okole River
07-APU	103	0.995	0.005	B6	B	1.000	0.000	1	Okole River
07-APU	104	0.991	0.009	B1	B	0.914	0.086	3	Okole River
07-APU	105	0.967	0.033	B1	B	0.917	0.083	1	Okole River
08-OCA	106	0.988	0.012	A1	A	0.992	0.008	6	Okole River
08-OCA	107	0.971	0.029	B6	B	0.968	0.032	4	Okole River
08-OCA	108	0.993	0.007	B6	B	0.981	0.019	4	Okole River
08-OCA	109	0.898	0.102	A2	A	0.999	0.001	4	Okole River
08-OCA	110	0.989	0.011	A1	A	1.000	0.000	4	Okole River
08-OCA	111	0.993	0.007	A2	A	1.000	0.000	6	Okole River
08-OCA	112	0.963	0.037	A4	A	1.000	0.000	1	Okole River
08-OCA	113	0.994	0.006	A2	A	1.000	0.000	6	Okole River
08-OCA	114	0.974	0.026	A1	A	1.000	0.000	1	Okole River
08-OCA	115	0.988	0.012	A4	A	0.977	0.023	6	Okole River
08-OCA	116	0.957	0.043	B5	B	0.998	0.002	1	Okole River
08-OCA	117	0.985	0.015	A1	A	0.999	0.001	4	Okole River
08-OCA	118	0.950	0.050	A2	A	1.000	0.000	6	Okole River
08-OCA	119	0.996	0.004	A1	A	0.956	0.044	6	Okole River
08-OCA	120	0.960	0.040	A2	A	0.987	0.013	6	Okole River
08-OCA	121	0.995	0.005	B1	B	0.999	0.001	6	Okole River
08-OCA	122	0.736	0.264	A1	A	1.000	0.000	6	Okole River
08-OCA	123	0.975	0.025	B1	B	1.000	0.000	4	Okole River
08-OCA	124	0.994	0.006	B2	B	1.000	0.000	1	Okole River
08-OCA	125	0.986	0.014	B1	B	1.000	0.000	6	Okole River
09-UWA	126	0.008	0.992	A5	A	0.999	0.001	6	Okole River
09-UWA	127	0.005	0.995	A5	A	1.000	0.000	1	Okole River
09-UWA	128	0.010	0.990	C18	C	0.964	0.036	6	Okole River
09-UWA	129	0.031	0.969	A2	A	1.000	0.000	4	Okole River
09-UWA	130	0.005	0.995	C18	C	0.999	0.001	4	Okole River
09-UWA	131	0.004	0.996	A2	A	0.999	0.001	2	Okole River
09-UWA	132	0.007	0.994	C18	C	0.999	0.001	6	Okole River
09-UWA	133	0.031	0.969	A5	A	1.000	0.000	4	Okole River
09-UWA	134	0.009	0.991	A2	A	1.000	0.000	4	Okole River
09-UWA	135	0.008	0.992	A5	A	1.000	0.000	4	Okole River
09-UWA	136	0.026	0.974	C18	C	0.999	0.001	4	Okole River
09-UWA	137	0.014	0.986	C18	C	0.135	0.865	9	Okole River
09-UWA	138	0.004	0.996	N/A	N/A	0.999	0.001	6	Okole River
09-UWA	139	0.007	0.993	A5	A	0.080	0.920	9	Okole River
09-UWA	140	0.007	0.993	A5	A	0.056	0.944	8	Okole River

10-OCU	141	0.979	0.021	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	142	0.993	0.007	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	143	0.982	0.018	B5	B	1.000	0.000	1	Lake Kyoga
10-OCU	144	0.994	0.006	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	145	0.981	0.019	B7	B	0.977	0.023	3	Lake Kyoga
10-OCU	146	0.991	0.009	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	147	0.988	0.012	B1	B	1.000	0.000	1	Lake Kyoga
10-OCU	148	0.986	0.014	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	149	0.979	0.021	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	150	0.913	0.087	B5	B	1.000	0.000	5	Lake Kyoga
10-OCU	151	0.994	0.006	B1	B	0.999	0.001	6	Lake Kyoga
10-OCU	152	0.987	0.013	B1	B	0.886	0.114	6	Lake Kyoga
10-OCU	153	0.954	0.046	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	154	0.982	0.018	B5	B	1.000	0.000	5	Lake Kyoga
10-OCU	155	0.996	0.004	B1	B	1.000	0.000	1	Lake Kyoga
10-OCU	156	0.993	0.007	B1	B	1.000	0.000	6	Lake Kyoga
10-OCU	157	0.996	0.004	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	158	0.993	0.007	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	159	0.996	0.004	B1	B	1.000	0.000	5	Lake Kyoga
10-OCU	160	0.993	0.007	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	161	0.993	0.007	N/A	N/A	1.000	0.000	1	Lake Kyoga
11-OT	162	0.994	0.006	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	163	0.994	0.006	B1	B	1.000	0.000	1	Lake Kyoga
11-OT	164	0.995	0.005	B1	B	1.000	0.000	1	Lake Kyoga
11-OT	165	0.926	0.074	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	166	0.969	0.032	N/A	N/A	0.997	0.003	6	Lake Kyoga
11-OT	167	0.995	0.005	N/A	N/A	1.000	0.000	1	Lake Kyoga
11-OT	168	0.960	0.040	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	169	0.985	0.015	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	170	0.995	0.005	B1	B	1.000	0.000	5	Lake Kyoga
11-OT	171	0.995	0.005	N/A	N/A	1.000	0.000	1	Lake Kyoga
11-OT	172	0.995	0.005	N/A	N/A	1.000	0.000	5	Lake Kyoga
11-OT	173	0.988	0.012	N/A	N/A	1.000	0.000	5	Lake Kyoga
11-OT	174	0.969	0.031	B5	B	1.000	0.000	1	Lake Kyoga
12-BN	175	0.682	0.318	N/A	N/A	0.609	0.391	7	Lake Kyoga
12-BN	176	0.683	0.317	B1	B	0.796	0.204	7	Lake Kyoga
12-BN	177	0.573	0.427	B5	B	0.776	0.224	1	Lake Kyoga
12-BN	178	0.993	0.007	B5	B	0.851	0.149	7	Lake Kyoga
12-BN	179	0.986	0.015	B1	B	0.630	0.370	7	Lake Kyoga
12-BN	180	0.981	0.019	N/A	N/A	0.742	0.258	7	Lake Kyoga
12-BN	181	0.980	0.020	N/A	N/A	0.767	0.233	7	Lake Kyoga
12-BN	182	0.748	0.252	N/A	N/A	0.659	0.341	7	Lake Kyoga
12-BN	183	0.989	0.012	N/A	N/A	0.662	0.338	7	Lake Kyoga
12-BN	184	0.606	0.394	C18	C	0.674	0.326	7	Lake Kyoga
12-BN	185	0.988	0.012	N/A	N/A	0.690	0.310	7	Lake Kyoga
12-BN	186	0.837	0.163	N/A	N/A	0.680	0.320	7	Lake Kyoga
13-MF	187	0.067	0.933	A5	A	0.033	0.967	10	Lake Albert
13-MF	188	0.071	0.929	A5	A	0.047	0.953	10	Lake Albert
13-MF	189	0.020	0.980	A5	A	0.094	0.906	8	Lake Albert

13-MF	190	0.013	0.987	A5	A	0.055	0.945	9	Lake Albert
13-MF	191	0.004	0.996	A5	A	0.038	0.962	10	Lake Albert
13-MF	192	0.005	0.995	A5	A	0.055	0.945	10	Lake Albert
13-MF	193	0.049	0.951	A5	A	0.044	0.956	10	Lake Albert
13-MF	194	0.075	0.925	A13	A	0.042	0.958	10	Lake Albert
13-MF	195	0.025	0.975	A5	A	0.039	0.961	10	Lake Albert
13-MF	196	0.069	0.931	A5	A	0.025	0.975	10	Lake Albert
14-MS	197	0.084	0.916	C18	C	0.012	0.988	10	Lake Albert
14-MS	198	0.011	0.989	A7	A	0.015	0.985	10	Lake Albert
14-MS	199	0.010	0.990	C18	C	0.018	0.982	10	Lake Albert
14-MS	200	0.022	0.979	A7	A	0.133	0.867	9	Lake Albert
14-MS	201	0.011	0.989	A7	A	0.129	0.871	8	Lake Albert
14-MS	202	0.006	0.994	A7	A	0.000	1.000	10	Lake Albert
14-MS	203	0.009	0.991	A7	A	0.130	0.870	8	Lake Albert
14-MS	204	0.008	0.993	A7	A	0.000	1.000	10	Lake Albert
14-MS	205	0.015	0.985	A7	A	0.001	0.999	10	Lake Albert
14-MS	206	0.034	0.966	A7	A	0.054	0.946	9	Lake Albert
14-MS	207	0.014	0.986	A7	A	0.005	0.995	10	Lake Albert
15-KAF	208	0.005	0.995	A5	A	0.003	0.997	9	Kafu River
15-KAF	209	0.005	0.995	A5	A	0.000	1.000	10	Kafu River
15-KAF	210	0.011	0.989	N/A	N/A	0.089	0.911	8	Kafu River
15-KAF	211	0.046	0.954	A5	A	0.000	1.000	10	Kafu River
15-KAF	212	0.015	0.985	A5	A	0.000	1.000	10	Kafu River
15-KAF	213	0.026	0.974	A5	A	0.000	1.000	10	Kafu River
15-KAF	214	0.008	0.992	A5	A	0.000	1.000	10	Kafu River
15-KAF	215	0.010	0.990	A5	A	0.000	1.000	10	Kafu River
15-KAF	216	0.012	0.988	A5	A	0.000	1.000	10	Kafu River
15-KAF	217	0.008	0.992	A5	A	0.081	0.919	8	Kafu River
15-KAF	218	0.006	0.994	A5	A	0.076	0.924	8	Kafu River
15-KAF	219	0.006	0.994	A5	A	0.094	0.906	9	Kafu River
15-KAF	220	0.123	0.877	A2	A	0.060	0.940	8	Kafu River
15-KAF	221	0.020	0.980	A5	A	0.016	0.984	8	Kafu River
15-KAF	222	0.006	0.994	A5	A	0.053	0.947	8	Kafu River
15-KAF	223	0.011	0.989	A2	A	0.015	0.985	9	Kafu River
15-KAF	224	0.008	0.992	A5	A	0.000	1.000	10	Kafu River
15-KAF	225	0.022	0.978	A7	A	0.000	1.000	10	Kafu River
15-KAF	226	0.013	0.988	A5	A	0.000	1.000	10	Kafu River
15-KAF	227	0.021	0.979	A5	A	0.000	1.000	10	Kafu River
16-JN	228	0.004	0.996	N/A	N/A	0.000	1.000	10	Lake Victoria
16-JN	229	0.013	0.987	A5	A	0.000	1.000	10	Lake Victoria
16-JN	230	0.005	0.995	D1	D	0.000	1.000	10	Lake Victoria
16-JN	231	0.006	0.994	A5	A	0.000	1.000	10	Lake Victoria
16-JN	234	0.005	0.995	A5	A	0.000	1.000	10	Lake Victoria
17-NB	235	0.006	0.994	N/A	N/A	0.000	1.000	10	Lake Victoria
17-NB	236	0.007	0.993	D2	D	0.000	1.000	10	Lake Victoria
17-NB	237	0.017	0.983	D2	D	0.000	1.000	10	Lake Victoria
17-NB	238	0.007	0.993	C18	C	0.000	1.000	10	Lake Victoria
17-NB	239	0.008	0.992	N/A	N/A	0.000	1.000	10	Lake Victoria
18-WAM	240	0.110	0.890	C18	C	0.000	1.000	9	Lake Victoria

18-WAM 241	0.010	0.990	D2	D	0.000	1.000	9	Lake Victoria
18-WAM 242	0.008	0.992	D1	D	0.000	1.000	10	Lake Victoria
18-WAM 243	0.008	0.993	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 244	0.009	0.991	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 245	0.013	0.987	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 246	0.007	0.993	C18	C	0.000	1.000	10	Lake Victoria
18-WAM 247	0.006	0.994	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 248	0.008	0.992	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 249	0.007	0.993	C18	C	0.000	1.000	9	Lake Victoria
18-WAM 250	0.015	0.985	C18	C	0.002	0.998	9	Lake Victoria
18-WAM 251	0.006	0.994	C18	C	0.001	0.999	9	Lake Victoria
18-WAM 252	0.028	0.972	D2	D	0.003	0.997	9	Lake Victoria
18-WAM 253	0.006	0.994	C1	C	0.000	1.000	10	Lake Victoria

Table S9. Summary of FINESTRUCTURE v. 2.0.7 (Lawson et al. 2012) cluster assignment (Figure 6). Count of individuals from each watershed assigned to ten FINESTRUCTURE v. 2.0.7 (Lawson et al. 2012) clusters numbered 1-10 (c1, c2, c3, c4, c5, c6, c7, c8, c9, and c10).

	Count of each fineSTRUCTURE cluster									
	c1	**c2**	**c3**	**c4**	**c5**	**c6**	**c7**	**c8**	**c9**	**c10**
Albert Nile	0	**13**	**17**	0	0	0	**1**	0	0	0
Achwa River	**3**	**2**	**1**	**26**	**2**	**5**	0	0	0	0
Okole River	**13**	**1**	**11**	**18**	**1**	**15**	0	**1**	**2**	0
Lake Kyoga	**7**	0	**1**	0	**18**	**3**	**4**	0	0	0
Lake Albert	0	0	0	0	0	0	0	**8**	**6**	**33**
Kafu River	0	0	0	0	0	0	0	**7**	**5**	**18**
Lake Victoria	0	0	0	0	0	0	0	0	**11**	**10**

Table S10. Summary statistics from 16 microsatellite loci and DNA sequences from a 490 bp mtDNA COI-COII gene fragment for samples grouped by **(a)** sampling site, and **(b)** watershed: Number of individuals genotyped (N), allelic richness (AR), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), number of sequences (N), number of haplotypes (Nh), haplotype diversity (Hd), and nucleotide diversity (Nd). F_{IS} values are marked with ^{1} if significantly different from zero with p<0.02 based on 1000 bootstrap replicates, marked with ^{1,2} if p<0.02 after Benjamini-Hochberg correction for multiple testing, or ^{1,2,3} if p<0.02 after Bonferroni correction for multiple testing.

(a)

	Microsatellite Results					mtDNA Results			
Site Code	**N**	**AR**	**H_O**	**H_E**	**F_{IS}**	**N**	**Nh**	**Hd**	**Nd**
01-DUK	20	3.59	0.625	0.700	$0.123^{1,2,3}$	20	5	0.368	0.0018
02-ORB	12	3.20	0.516	0.630	$0.180^{1,2,3}$	12	3	0.545	0.0012
03-NGO	12	3.32	0.593	0.646	0.061	12	3	0.439	0.0016
04-BOL	19	3.30	0.568	0.627	$0.096^{1,2}$	14	3	0.473	0.0012
05-CHU	13	3.22	0.588	0.607	0.024	13	5	0.731	0.0023
06-ACA	18	3.34	0.569	0.648	$0.144^{1,2,3}$	17	6	0.750	0.0025
07-APU	10	2.95	0.530	0.590	0.117^{1}	10	3	0.511	0.0011
08-OCA	20	3.27	0.556	0.631	$0.120^{1,2,3}$	20	7	0.842	0.0031
09-UWA	15	3.18	0.635	0.619	0.016	14	3	0.692	0.0071
10-OCU	20	3.04	0.513	0.584	0.135	20	3	0.353	0.0017
11-OT	14	2.91	0.607	0.569	-0.068	8	2	0.250	0.0015
12-BN	12	2.99	0.454	0.590	$0.211^{1,2,3}$	5	3	0.800	0.0053
13-MF	10	3.28	0.572	0.655	0.122^{1}	10	2	0.200	0.0014
14-MS	11	2.74	0.534	0.549	0.040	11	2	0.327	0.0047
15-KAF	20	2.81	0.505	0.537	0.070	19	3	0.292	0.0018
16-JN	5	2.38	0.500	0.526	0.043	6	3	0.600	0.0054
17-NB	5	2.47	0.546	0.552	0.012	3	2	0.667	0.0041
18-WAM	14	2.43	0.464	0.468	0.006	14	5	0.593	0.0028

(b)

	Microsatellite Results					mtDNA Results			
Watershed	**N**	**AR**	**H_O**	**H_E**	**F_{IS}**	**N**	**Nh**	**Hd**	**Nd**
Albert Nile	32	5.37	0.570	0.676	$0.145^{1,2,3}$	32	6	0.435	0.0010
Achwa River	45	5.26	0.584	0.636	$0.082^{1,2,3}$	39	8	0.609	0.0017
Okole River	63	5.58	0.573	0.685	$0.175^{1,2,3}$	61	10	0.851	0.0043
Lake Kyoga	46	4.76	0.525	0.617	$0.144^{1,2,3}$	33	4	0.403	0.0014
Lake Albert	21	4.80	0.553	0.638	$0.129^{1,2,3}$	21	4	0.652	0.0045
Kafu River	20	3.93	0.505	0.537	0.070	19	3	0.292	0.0018
Lake Victoria	24	3.68	0.482	0.501	0.048	23	7	0.771	0.0075
Overall	251	4.77	0.549	0.691	0.145	22	25	0.840	0.0047

MOLECULAR ECOLOGY

Table S11. Microsatellite pairwise F_{ST} (Wright 1951; Weir and Cockerham 1984) below the diagonal, and *p*-values above the diagonal from 16 loci for samples grouped by **(a)** sampling site, and **(b)** watershed, as implemented in FSTAT 2.9.3 (Goudet 1995). Significance of F_{ST} was estimated using 1000 randomizations in FSTAT.

(A)	**01- DUK**	**02- ORB**	**03- NGO**	**04- BOL**	**05- CHU**	**06- ACA**	**07- APU**	**08- OCA**	**09- UWA**	**10- OCU**	**11- OT**	**12- BN**	**13- MF**	**14- MS**	**15- KAF**	**16- JN**	**17- NB**	**18- WAM**
01-DUK		0.014	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
02-ORB	0.002		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
03-NGO	0.013	0.022		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001
04-BOL	0.033	0.055	0.028		0.015	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
05-CHU	0.032	0.035	0.039	0.013		0.060	0.438	0.002	<0.001	<0.001	0.019	0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001
06-ACA	0.029	0.043	0.059	0.021	0.016		0.020	0.069	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001
07-APU	0.046	0.057	0.055	0.010	0.004	0.017		<0.001	<0.001	<0.001	0.001	0.001	<0.001	0.001	<0.001	0.002	0.001	<0.001
08-OCA	0.057	0.073	0.082	0.035	0.043	0.003	0.036		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
09-UWA	0.137	0.175	0.185	0.195	0.192	0.174	0.203	0.194		<0.001	<0.001	<0.001	0.005	<0.001	<0.001	0.001	<0.001	<0.001
10-OCU	0.076	0.094	0.096	0.060	0.049	0.052	0.058	0.046	0.210		0.128	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
11-OT	0.059	0.066	0.082	0.042	0.015	0.049	0.037	0.075	0.225	0.019		0.001	<0.001	0.001	<0.001	0.002	0.001	<0.001
12-BN	0.119	0.144	0.123	0.122	0.123	0.091	0.141	0.117	0.179	0.123	0.143		0.001	0.006	0.001	0.006	0.002	0.001
13-MF	0.094	0.141	0.136	0.156	0.156	0.129	0.156	0.149	0.034	0.169	0.192	0.150		0.001	0.001	0.018	0.002	<0.001
14-MS	0.151	0.194	0.205	0.228	0.225	0.206	0.244	0.230	0.121	0.224	0.250	0.212	0.096		<0.001	0.005	0.003	0.001
15-KAF	0.150	0.182	0.189	0.193	0.166	0.174	0.211	0.195	0.129	0.198	0.188	0.220	0.113	0.146		0.003	0.001	<0.001
16-JN	0.149	0.188	0.192	0.191	0.190	0.185	0.220	0.206	0.119	0.242	0.237	0.216	0.084	0.239	0.149		0.037	0.003
17-NB	0.141	0.184	0.214	0.225	0.201	0.189	0.244	0.218	0.081	0.268	0.251	0.183	0.084	0.235	0.213	0.071		0.393
18-WAM	0.187	0.240	0.212	0.217	0.224	0.207	0.227	0.236	0.159	0.253	0.261	0.221	0.139	0.289	0.238	0.096	-0.021	

(B)	**Albert Nile**	**Achwa River**	**Okole River**	**Lake Kyoga**	**Lake Albert**	**Kafu River**	**Lake Victoria**
Albert Nile		0.002	0.002	0.002	0.002	0.002	0.002
Achwa River	0.025		0.002	0.002	0.002	0.002	0.002
Okole River	0.035	0.029		0.002	0.002	0.002	0.002
Lake Kyoga	0.063	0.040	0.037		0.002	0.002	0.002
Lake Albert	0.119	0.158	0.095	0.155		0.002	0.002
Kafu River	0.155	0.170	0.124	0.168	0.098		0.002
Lake Victoria	0.186	0.190	0.145	0.205	0.159	0.208	

(C)	**NW**	**“b”**	**“a”**	**NE**	**W**	**“c”**	**“d”**	**S**
Northwest		0.002	0.002	0.002	0.002	0.002	0.002	0.002
Zone “b”	0.022		0.002	0.002	0.002	0.002	0.002	0.002
Zone “a”	0.073	0.060		0.002	0.002	0.002	0.002	0.002
Northeast	0.043	0.020	0.113		0.002	0.002	0.002	0.002
West	0.067	0.063	0.040	0.104		0.002	0.002	0.002
“c”	0.081	0.069	0.065	0.105	0.058		0.002	0.002
“d”	0.057	0.038	0.094	0.071	0.090	0.102		0.002
South	0.087	0.063	0.101	0.126	0.104	0.107	0.086	

Table S12. Results from the MIGRAINE analysis for each lineage. We tested for population size change using the "OnePopVarSize" model (Peery et al. 2012; Leblois et al. 2014) under a generalized stepwise mutation model. Population size estimates were converted from estimates of diversity (θ and θ_{anc}) into effective population size (N and N_{anc}) using the equations (current: $\theta=2N\mu$, and ancestral: $\theta_{anc}=2N_{anc}\mu$) and scaled time (D/2N and Dμ) into time in generations (D) and time in years (T). We used a generation time of 45 days (Krafsur et al. 2009) and a mutation rate of 1 x 10^{-4} per generation per locus.

Sample	**θ_{anc}**	**θ**	**Demographic change**	**Ne_{anc}**	**Ne**
Northwest (Albert Nile)	3.049 [0.559-4.024]	2.253 [1.029-8.434]	Non-significant	7622.5 [1397.5-10060]	5632.5 [2572.5-21085]
Northeast (Lake Kyoga)	2.176 [0.129-2.934]	2.402 [1.263-5.013]	Non-significant	5440 [322.5-7335]	6005 [3157.5-12532.5]
West (Lake Albert)	1.012 [0.205-1.046]	2.253 [2.24-2.752]	Expansion	2530 [512.5-2615]	5632.5 [5600-6880]
South (Lake Victoria)	0.444 [0.358-2.393]	0.986 [0.855-1.056]	Non-significant	1110 [895-5982.5]	2465 [2137.5-2640]

Table S13. Results from the microsatellite ABC analysis on northern Uganda implemented in DIYABC (Cornuet et al., 2008) based on the alternative topology 2b (Figure 3). **(a)** Posterior probabilities of competing scenarios 1-3 by the polychotomic weighted logistic regression method and lower and upper 95% confidence interval with notation “probability (lower,upper)” for microsatellites. **(b)** Parameter estimates based on the winning Scenario 2 including the mean, 95% confidence interval (CI), median and mode of the posterior distribution, and the description of each parameter estimated. Time estimates (t1, t2, t3, and t4) are expressed in years (yrs), effective population size estimates (N1, N2, N3, N4, N5) are expressed in number of breeding individuals. We also report estimates of the microsatellite mutation rate (μ) and geometric distribution of change in repeat length (P). **(c)** DIYABC posterior checking under **Scenario 2** showing the simulated summary statistics, their variance, and the proportion of the simulated values that were less than the observed real data. Abbreviations: NAL=number of alleles, HET=heterozygosity, MGW=M-index (Garza and Williamson, 2001; Excoffier et al., 2005), F_{ST}=Weir and Cockerham (1984) genetic distance, NW=northwest, NE=northeast, “b”=admixture zone “b”, W=west, S=south.

(a)

Dataset	Scenario 1	Scenario 2	Scenario 3	Error	Best Scenario
Microsatellites	0.001 (0.000,0.002)	0.999 (0.998,1.000)	0.000 (0.000,0.000)	0.000	2

(b)

Parameter	Mean	CI	Median	Mode	Description
t1	61	16–107	61	59	NE vs b” divergence time
t2	82	32–129	83	92	NW vs NE divergence time
t3	23,250	20,125–32,875	21,750	20,000	W vs S divergence time
t4	391,250	343,750–451,250	386,250	345,000	NW vs W divergence time
N1	23,600	16,800–28,700	24,100	24,900	Ne of NW (Albert Nile)
N2	17,800	6,860–28,400	17,800	17,300	Ne of “b” (Okole River)
N3	9,940	2,950–22,800	8,350	6,010	Ne of NE (Lake Kyoga)
N4	12,200	5,020–21,600	11,600	11,200	Ne of W (Lake Albert)
N5	8,780	3,700–15,900	8,160	7,430	Ne of S (Lake Victoria)
μ	1.18E-04	1.00–1.58E-04	1.10E-04	1.00E-04	Mutation rate
P	0.2090	0.1340–0.2780	0.2110	0.2110	Geometric distribution of change in repeat

(c)

Statistic	Simulated value	Variance	Proportion simulated < real	Statistic	Simulated value	Variance	Proportion simulated < real
NAL NW	5.937	0.808	0.0075	F_{ST} NW vs “b”	0.034	0.000	0.9110
NAL ”b”	5.875	0.923	0.0045	F_{ST} NW vs NE	0.053	0.000	0.9500
NAL NE	5.562	0.959	0.0170	F_{ST} NW vs W	0.144	0.002	0.0010
NAL W	3.500	0.678	0.0175	F_{ST} NW vs S	0.189	0.002	0.0140
NAL S	3.437	0.728	0.0215	F_{ST} “b” vs NE	0.033	0.000	0.8570
HET NW	0.700	0.001	0.0305	F_{ST} “b” vs W	0.203	0.002	0.0730
HET ”b”	0.634	0.001	0.0020	F_{ST} ”b” vs S	0.204	0.002	0.0250
HET NE	0.631	0.002	0.0085	F_{ST} NE vs W	0.188	0.002	0.0210
HET W	0.552	0.005	0.0495	F_{ST} NE vs S	0.199	0.002	0.0135
HET S	0.470	0.005	0.0195	F_{ST} W vs S	0.269	0.004	0.3365
MGW NW	0.725	0.005	0.3805				
MGW ”b”	0.695	0.006	0.2145				
MGW NE	0.681	0.006	0.2930				
MGW W	0.528	0.007	0.0155				
MGW S	0.593	0.007	0.0350				

Table S14. Results from the ddRAD SNP based ABC analysis on northern Uganda implemented in DIYABC (Cornuet et al., 2008). For the ddRAD SNPs, the only method available was a simplified and efficient algorithm that does not require or accept any parameterization of the mutation model (Cornuet et al. 2014) beyond a minor allele frequency (MAF), which we restricted as little as possible to the MAF that equates to 0.05 (DIYABC flag <MAF=hudson>). Unfortunately, this simplified mutation model available in DIYABC did not provide a good match of the modeled data with the real data (Figure S14), and could not be improved because of no user choices, thus these results are unreliable, and are presented only in the interest of completeness. The tables display posterior probabilities of competing scenarios 1-3 from the ddRAD SNP analysis by the polychotomic weighted logistic regression method and lower and upper 95% confidence interval with notation "probability (lower,upper)", and estimates from DIYABC (Cornuet et al., 2008), with priors based on the winning Scenario 2 with **(a)** the main topology 2a, and **(b)** the alternative topology 2b. We report the mean, median and mode, and description of each parameter estimated from simulated data in DIYABC (Cornuet et al., 2008) under the scenario with the highest probability, Scenario 2 (Table 3). We report five effective population size (Ne) estimates, and four time estimates (t1.1, t1.2, t3, and t4; Figure 3) expressed in years assuming eight *Glossina fuscipes fuscipes* generations per year (Krafsur et al., 2009. See Figure 1 for identification of the watersheds, Figure 3 for schematics of the three scenarios, Table S3 for details of the priors used, and Table 3 for results from the same analyses for the microsatellite dataset.

(a) Topology 2a

Dataset	Scenario 1	Scenario 2	Scenario 3	Error	Best Scenario
ddRAD SNPs	0.000 [0.000–1.000]	1.000 [1.000–1.000]	0.000 [0.000–1.000]	0.000	2

Parameter	Mean	Median	Mode	Description
t1.1	135	135	135	Northeast / admixture zone "b" divergence
t1.2	135	135	135	Northwest / northeast divergence
t3	20,000	20,000	20,000	West / south divergence
t4	340,000	340,000	340,000	West / northwest divergence
N1	30,0000	30,000	30,000	Ne of northwest lineage
N2	100	100	100	Ne of admixture zone "b"
N3	100	100	100	Ne of northeast lineage
N4	30,000	30,000	30,000	Ne of west lineage
N5	30,000	30,000	30,000	Ne of south lineage

(b) Topology 2b

Dataset	Scenario 1	Scenario 2	Scenario 3	Error	Best Scenario
ddRAD SNPs	0.000 [0.000,1.000]	1.000 [1.000,1.000]	0.000 [0.000,1.000]	0.000	2

Parameter	Mean	Median	Mode	Description
t1.1	135	135	135	Northeast / admixture zone "b" divergence
t1.2	135	135	135	Northwest / northeast divergence
t3	20,000	20,000	20,000	West / south divergence
t4	340,000	340,000	340,000	West / northwest divergence
N1	30,0000	30,000	30,000	Ne of northwest lineage
N2	30,000	30,000	30,000	Ne of admixture zone "b"
N3	100	100	100	Ne of northeast lineage
N4	30,000	30,000	30,000	Ne of west lineage
N5	30,000	30,000	30,000	Ne of south lineage

Table S15. F_{ST} estimates greater than the null distribution (p-value < 0.02) across each of the four admixture zones **(a)** “a” between the northwest and west, **(b)** “b” between the northwest and northeast, and **(c)** “c” between the west and south, displaying SNP name, Scaffold, Position, heterozygosity (HE), FST, empirical q-values, and p-values. SNPs with a p-value < 0.02 across both zones "a" and "b" marked *, SNPs with a p-value < 0.02 across both zones "a" and "c" marked †. Admixture zone “d” did not contain any outliers. F_{ST} estimates and outlier tests were performed in OutFLANK v0.2 (Whitlock and Lotterhos, 2015). Note that even in the top outliers q-values were not significant, likely due to low sample sizes.

(a)

SNP	Scaffold	Position	HE	FST	q-values	p-values
204332_53	Scaffold278	92607	0.4600	0.8784	0.9697	0.0114
159994_38	Scaffold224	92931	0.4521	0.8686	0.9697	0.0118
78595_66	Scaffold148	229632	0.4877	0.8577	0.9697	0.0126
32715_7	Scaffold1119	1187	0.4527	0.8322	0.9697	0.0142
196048_48	Scaffold268	5102	0.4998	0.8279	0.9697	0.0145
16810_7	Scaffold1029	29356	0.4982	0.8230	0.9697	0.0150
303574_36	Scaffold419	105265	0.4200	0.8197	0.9697	0.0151
406475_29	Scaffold629	80329	0.4531	0.8167	0.9697	0.0154
19663_16	Scaffold1046	26392	0.4423	0.8146	0.9697	0.0156
448690_6	Scaffold74	155468	0.4991	0.8087	0.9697	0.0160
78583_37	Scaffold148	219791	0.4931	0.8070	0.9697	0.0162
391939_9	Scaffold597	20866	0.4377	0.8062	0.9697	0.0163
241520_62	Scaffold322	285918	0.4764	0.8036	0.9697	0.0163
383602_14	Scaffold576	115443	0.4584	0.8048	0.9697	0.0164
269896_16†	Scaffold368	279432	0.4422	0.8017	0.9697	0.0166
201395_64	Scaffold274	21592	0.4980	0.7979	0.9697	0.0168
228722_16	Scaffold304	141786	0.5000	0.7947	0.9697	0.0171
502652_20	Scaffold969	52687	0.4998	0.7913	0.9697	0.0175
492051_17*	Scaffold916	34536	0.5000	0.7880	0.9697	0.0178
449863_18	Scaffold750	14030	0.4774	0.7855	0.9697	0.0178
474540_24	Scaffold841	73663	0.4993	0.7876	0.9697	0.0179
243855_7	Scaffold328	170538	0.4254	0.7777	0.9697	0.0184
118616_25	Scaffold185	139086	0.4906	0.7796	0.9697	0.0184
271472_15	Scaffold36	46337	0.4200	0.7735	0.9697	0.0189
379407_40	Scaffold566	45008	0.4288	0.7711	0.9697	0.0195
379409_11	Scaffold566	45056	0.4288	0.7711	0.9697	0.0195
274230_62	Scaffold374	7620	0.4288	0.7680	0.9697	0.0195
87115_19	Scaffold154	97489	0.4886	0.7671	0.9697	0.0196
460746_4	Scaffold795	17873	0.4886	0.7671	0.9697	0.0196
13033_36	Scaffold1004	13111	0.4998	0.7657	0.9697	0.0197
470412_14	Scaffold825	28170	0.4132	0.7619	0.9697	0.0200

(b)

SNP	Scaffold	Position	HE	FST	q-values	p-values
369504_12	Scaffold541	95299	0.4819	0.8220	0.7577	0.0002
250948_8	Scaffold339	212404	0.4988	0.6567	0.7577	0.0010
426423_15	Scaffold687	11087	0.4934	0.6220	0.7577	0.0015
387032_30	Scaffold584	124925	0.4995	0.6224	0.7577	0.0015
172870_6	Scaffold238	441981	0.4775	0.6205	0.7577	0.0015

229625_20	Scaffold305	356608	0.4995	0.6128	0.7577	0.0017
357276_58	Scaffold515	114979	0.4942	0.6093	0.7577	0.0017
492051_17*	Scaffold916	34536	0.4995	0.6089	0.7577	0.0017
330996_43	Scaffold468	111258	0.4999	0.6038	0.7577	0.0019
391154_29	Scaffold594	102293	0.4586	0.5998	0.7577	0.0019
32954_56	Scaffold111	288981	0.4919	0.5901	0.7577	0.0021
273423_5	Scaffold373	106447	0.4819	0.5797	0.7577	0.0024
87440_4	Scaffold155	329956	0.4200	0.5723	0.7577	0.0025
451025_44	Scaffold758	27041	0.4830	0.5700	0.7577	0.0026
431667_62	Scaffold69	98041	0.3866	0.5520	0.7577	0.0030
236745_6	Scaffold316	136935	0.4999	0.5502	0.7577	0.0031
325923_14	Scaffold458	87390	0.4444	0.5441	0.7577	0.0033
429194_45	Scaffold692	94930	0.4957	0.5411	0.7577	0.0035
170959_20	Scaffold236	154134	0.4005	0.5404	0.7577	0.0035
171092_24	Scaffold236	221928	0.3935	0.5240	0.7577	0.0042
488333_32	Scaffold8	1955342	0.3711	0.5197	0.7577	0.0043
122124_18	Scaffold189	75525	0.4314	0.5197	0.7577	0.0043
429360_6	Scaffold693	83913	0.3750	0.5154	0.7577	0.0045
309869_34	Scaffold42	1024430	0.4237	0.5111	0.7577	0.0046
135969_16	Scaffold1	2139752	0.4773	0.5091	0.7577	0.0048
412494_23	Scaffold646	101966	0.4826	0.5014	0.7577	0.0050
212192_5	Scaffold288	39111	0.4999	0.5030	0.7577	0.0050
1739_27	JFJR01009825	11413	0.4914	0.5016	0.7577	0.0051
176069_66	Scaffold240	331932	0.4898	0.5009	0.7577	0.0052
345557_21	Scaffold497	13242	0.4989	0.4986	0.7577	0.0053
85493_19	Scaffold152	543423	0.4636	0.4959	0.7577	0.0054
438783_10	Scaffold716	32859	0.4861	0.4981	0.7577	0.0055
359458_46	Scaffold51	424477	0.4658	0.4932	0.7577	0.0057
287563_23	Scaffold398	163793	0.4545	0.4928	0.7577	0.0057
479784_15	Scaffold868	35479	0.4641	0.4826	0.7577	0.0059
87252_45	Scaffold155	202087	0.3240	0.4820	0.7577	0.0062
121680_28	Scaffold189	203382	0.3825	0.4813	0.7577	0.0062
121269_21	Scaffold188	408363	0.5000	0.4848	0.7577	0.0062
487211_29	Scaffold8	1115418	0.3594	0.4816	0.7577	0.0062
8378_13	JFJR01013077	680	0.4624	0.4826	0.7577	0.0063
313752_5	Scaffold435	60090	0.4907	0.4836	0.7577	0.0063
450712_7	Scaffold756	30753	0.4512	0.4791	0.7577	0.0063
361672_62	Scaffold525	196224	0.4110	0.4774	0.7577	0.0064
456292_4	Scaffold776	43261	0.4144	0.4821	0.7577	0.0065
435284_41	Scaffold704	3137	0.4114	0.4748	0.7577	0.0066
488896_52	Scaffold8	308748	0.3947	0.4718	0.7577	0.0067
90588_24	Scaffold159	39972	0.4666	0.4760	0.7577	0.0069
5742_37	JFJR01012818	18784	0.3941	0.4709	0.7577	0.0070
536312_17	Scaffold18	439761	0.4936	0.4667	0.7577	0.0071
284388_41	Scaffold390	152026	0.4835	0.4696	0.7577	0.0072
419792_12	Scaffold668	52160	0.4837	0.4687	0.7577	0.0072
429290_41	Scaffold693	35301	0.4699	0.4663	0.7577	0.0072
173985_56	Scaffold23	1318781	0.4213	0.4648	0.7577	0.0075
410158_61	Scaffold63	133244	0.4938	0.4624	0.7577	0.0075
358894_23	Scaffold51	1084874	0.4043	0.4639	0.7577	0.0076

171114_26	Scaffold236	232584	0.4043	0.4639	0.7577	0.0076
66008_35	Scaffold138	142438	0.3529	0.4544	0.7577	0.0078
438165_27	Scaffold712	85993	0.4794	0.4614	0.7577	0.0080
442150_9	Scaffold729	109563	0.4901	0.4594	0.7577	0.0080
472110_16	Scaffold832	20427	0.4999	0.4542	0.7577	0.0081
382468_32	Scaffold571	38770	0.4213	0.4579	0.7577	0.0081
248433_15	Scaffold334	172184	0.3673	0.4499	0.7577	0.0085
278539_58	Scaffold381	172261	0.4005	0.4505	0.7577	0.0088
262340_8	Scaffold355	26815	0.4409	0.4489	0.7577	0.0089
412640_14	Scaffold646	68099	0.4861	0.4475	0.7577	0.0090
99562_27	Scaffold167	213357	0.4737	0.4464	0.7577	0.0091
127170_64	Scaffold194	247329	0.3181	0.4403	0.7577	0.0095
504526_7	Scaffold97	152374	0.4966	0.4406	0.7577	0.0095
267418_55	Scaffold362	37089	0.4706	0.4400	0.7577	0.0096
423783_43	Scaffold67	135491	0.4999	0.4387	0.7577	0.0096
494521_28	Scaffold927	24705	0.4444	0.4366	0.7577	0.0100
817_26	JFJR01008464	51452	0.4318	0.4362	0.7577	0.0100
381978_16	Scaffold56	959900	0.3829	0.4393	0.7577	0.0100
464211_39	Scaffold7	230519	0.3343	0.4354	0.7577	0.0101
157346_57	Scaffold221	182609	0.4734	0.4345	0.7577	0.0103
380187_30	Scaffold569	16925	0.3200	0.4331	0.7577	0.0104
47698_21	Scaffold122	184626	0.4725	0.4273	0.7577	0.0107
317298_52	Scaffold43	932210	0.4852	0.4281	0.7577	0.0109
365309_11	Scaffold532	53116	0.3143	0.4235	0.7577	0.0115
215736_7	Scaffold292	19287	0.4012	0.4236	0.7577	0.0117
505584_34	Scaffold984	23317	0.2965	0.4238	0.7577	0.0118
184621_36	Scaffold251	77729	0.2965	0.4238	0.7577	0.0118
167626_44	Scaffold231	164189	0.3254	0.4190	0.7577	0.0119
476145_5	Scaffold84	688082	0.4745	0.4165	0.7577	0.0119
350659_46	Scaffold500	131562	0.4527	0.4180	0.7577	0.0119
349783_53	Scaffold4	356273	0.4620	0.4173	0.7577	0.0123
29331_38	Scaffold10	168463	0.4426	0.4150	0.7577	0.0123
227975_47	Scaffold302	301031	0.4698	0.4131	0.7577	0.0123
324990_29	Scaffold456	230029	0.4297	0.4138	0.7577	0.0126
434589_8	Scaffold700	71819	0.3863	0.4174	0.7577	0.0126
275659_34	Scaffold378	137177	0.3380	0.4078	0.7577	0.0130
269805_34	Scaffold368	217355	0.4224	0.4099	0.7577	0.0132
62217_46	Scaffold1346	1764	0.4870	0.4120	0.7577	0.0133
392872_27	Scaffold59	267407	0.3897	0.4102	0.7577	0.0133
150087_59	Scaffold214	242478	0.3275	0.4076	0.7577	0.0134
234161_15	Scaffold311	174221	0.2919	0.4052	0.7577	0.0134
161217_18	Scaffold226	36737	0.2919	0.4110	0.7577	0.0134
309308_63	Scaffold428	44951	0.4592	0.4022	0.7577	0.0137
401847_48	Scaffold614	66868	0.4955	0.4031	0.7577	0.0137
286034_54	Scaffold394	221511	0.3221	0.4055	0.7577	0.0138
177452_4	Scaffold242	49705	0.3163	0.4037	0.7577	0.0140
177409_17	Scaffold242	424673	0.3163	0.4037	0.7577	0.0140
14179_16	Scaffold100	681334	0.4907	0.4023	0.7577	0.0142
223670_4	Scaffold2	1770151	0.3710	0.4014	0.7577	0.0143
405132_21	Scaffold623	67813	0.4857	0.3997	0.7577	0.0144

339185_35	Scaffold483	195611	0.4989	0.3980	0.7577	0.0147
212139_33	Scaffold288	354297	0.4289	0.4030	0.7577	0.0147
484709_60	Scaffold88	667768	0.4005	0.3951	0.7577	0.0148
171093_12	Scaffold236	221989	0.3407	0.4024	0.7577	0.0149
415174_61	Scaffold650	70871	0.4370	0.3955	0.7577	0.0149
280957_15	Scaffold386	112785	0.3291	0.3942	0.7577	0.0152
94487_13	Scaffold161	331707	0.3505	0.3967	0.7577	0.0153
503535_27	Scaffold96	767420	0.4717	0.3903	0.7577	0.0155
199932_44	Scaffold271	346142	0.4924	0.3889	0.7577	0.0155
436608_27	Scaffold70	359567	0.3264	0.3935	0.7577	0.0155
425370_8	Scaffold681	83075	0.4545	0.3976	0.7577	0.0157
455963_37	Scaffold774	51679	0.3594	0.3948	0.7577	0.0157
171086_7	Scaffold236	218279	0.4163	0.3928	0.7577	0.0158
250245_23	Scaffold337	351612	0.3006	0.3928	0.7577	0.0158
1783_15	JFJR01009825	30943	0.3122	0.3931	0.7577	0.0160
377724_6	Scaffold560	155024	0.4550	0.3896	0.7577	0.0161
158650_21	Scaffold222	454637	0.4999	0.3907	0.7577	0.0161
285116_6	Scaffold392	183282	0.4617	0.3889	0.7577	0.0161
132495_10	Scaffold19	1532359	0.4541	0.3890	0.7577	0.0162
5097_5	JFJR01012812	58035	0.2813	0.3905	0.7577	0.0162
412401_10	Scaffold645	46538	0.4478	0.3898	0.7577	0.0163
195776_40	Scaffold268	273433	0.3011	0.3844	0.7577	0.0164
171314_42	Scaffold236	333801	0.3070	0.3921	0.7577	0.0164
202486_29	Scaffold276	162955	0.3070	0.3897	0.7577	0.0164
484347_16	Scaffold88	38738	0.3070	0.3872	0.7577	0.0164
118504_5	Scaffold184	556868	0.4895	0.3879	0.7577	0.0166
195552_23	Scaffold268	143334	0.4848	0.3850	0.7577	0.0167
287274_12	Scaffold397	248000	0.4265	0.3866	0.7577	0.0167
442721_45	Scaffold72	348627	0.4807	0.3780	0.7577	0.0177
106966_11	Scaffold173	169931	0.3906	0.3787	0.7577	0.0177
375196_58	Scaffold554	47703	0.3143	0.3806	0.7577	0.0177
51721_6	Scaffold1264	1004	0.3314	0.3779	0.7577	0.0178
325615_38	Scaffold458	11365	0.3254	0.3808	0.7577	0.0179
486896_37	Scaffold89	783654	0.3711	0.3790	0.7577	0.0180
55039_68	Scaffold129	365714	0.3466	0.3784	0.7577	0.0182
207755_4	Scaffold280	93466	0.3624	0.3815	0.7577	0.0182
212776_6	Scaffold289	347074	0.3750	0.3771	0.7577	0.0183
88716_4	Scaffold157	237040	0.4658	0.3763	0.7577	0.0183
56837_41	Scaffold12	1955044	0.3665	0.3728	0.7577	0.0184
52011_16	Scaffold126	321531	0.4866	0.3718	0.7577	0.0184
152644_11	Scaffold218	130543	0.3314	0.3755	0.7577	0.0184
443986_45	Scaffold731	52656	0.4647	0.3769	0.7577	0.0186
237426_30	Scaffold317	336207	0.4765	0.3768	0.7577	0.0188
326567_65	Scaffold45	1161412	0.4100	0.3712	0.7577	0.0188
378245_8	Scaffold562	174492	0.3457	0.3718	0.7577	0.0189
494520_12	Scaffold927	24654	0.3466	0.3777	0.7577	0.0190
421862_32	Scaffold670	36745	0.3466	0.3765	0.7577	0.0190
147263_58	Scaffold20	779326	0.3466	0.3720	0.7577	0.0190
275219_5	Scaffold377	141988	0.3750	0.3794	0.7577	0.0190
182488_4	Scaffold24	185236	0.3980	0.3691	0.7577	0.0191

177786_47	Scaffold243	310941	0.3219	0.3672	0.7577	0.0192
422140_22	Scaffold672	118461	0.3788	0.3731	0.7577	0.0192
344904_7	Scaffold495	103315	0.2912	0.3753	0.7577	0.0194
171973_15	Scaffold237	306309	0.3254	0.3707	0.7577	0.0195
41706_6	Scaffold118	475827	0.4768	0.3691	0.7577	0.0199

(c)

SNP	Scaffold	Position	HE	FST	q-value	p-value
131309_22	Scaffold199	455215	0.4669	0.9404	0.8625	0.0072
320731_43	Scaffold448	87993	0.4800	0.9393	0.8625	0.0072
49747_6	Scaffold123	7704	0.4281	0.9265	0.8625	0.0077
171637_15	Scaffold236	87283	0.4935	0.9131	0.8625	0.0083
507716_9	Scaffold996	8400	0.4935	0.9131	0.8625	0.0083
441759_34	Scaffold726	74263	0.4592	0.9101	0.8625	0.0084
171606_66	Scaffold236	70730	0.4982	0.9075	0.8625	0.0085
376137_39	Scaffold559	102992	0.4728	0.8906	0.8625	0.0093
500014_8	Scaffold94	746726	0.4963	0.8848	0.8625	0.0096
461329_29	Scaffold79	141508	0.4506	0.8794	0.8625	0.0099
492213_70	Scaffold917	8143	0.4567	0.8762	0.8625	0.0100
170849_5	Scaffold236	111503	0.4935	0.8481	0.8625	0.0115
454140_62	Scaffold769	15203	0.4961	0.8433	0.8625	0.0118
370070_18	Scaffold543	244217	0.4853	0.8428	0.8625	0.0119
500867_37	Scaffold959	41145	0.4506	0.8095	0.8625	0.0141
405032_55	Scaffold623	132409	0.4669	0.8063	0.8625	0.0143
14550_10	Scaffold1012	37392	0.4669	0.8063	0.8625	0.0143
41304_9	Scaffold118	232784	0.5000	0.7997	0.8625	0.0147
485683_25	Scaffold899	48863	0.4885	0.7949	0.8625	0.0151
450471_30	Scaffold753	99496	0.4200	0.7827	0.8625	0.0160
501996_32	Scaffold961	7889	0.4200	0.7827	0.8625	0.0160
267227_65	Scaffold362	227931	0.4412	0.7761	0.8625	0.0167
234779_25	Scaffold312	218892	0.4412	0.7737	0.8625	0.0167
452994_60	Scaffold763	52329	0.4853	0.7735	0.8625	0.0169
264412_26	Scaffold359	313509	0.4739	0.7685	0.8625	0.0171
402460_63	Scaffold617	149510	0.4983	0.7674	0.8625	0.0175
269896_16†	Scaffold368	279432	0.4983	0.7651	0.8625	0.0175
55172_22	Scaffold129	43714	0.4980	0.7579	0.8625	0.0183
426726_6	Scaffold688	48002	0.4835	0.7568	0.8625	0.0184
200328_65	Scaffold272	243996	0.4541	0.7485	0.8625	0.0189
49066_31	Scaffold123	296971	0.4082	0.7505	0.8625	0.0189
435838_66	Scaffold707	60096	0.4604	0.7450	0.8625	0.0192
117188_17	Scaffold183	18727	0.4310	0.7373	0.8625	0.0199
441272_13	Scaffold725	18079	0.4898	0.7363	0.8625	0.0200

MOLECULAR ECOLOGY

Supplementary Python Code

```
#!/usr/bin/env python

"""
Name: treemix_tree_with_bootreps_NPS.py

Author: Michael G. Harvey and edited by norah.saarman@yale.edu to allow for migration
Date: 13 May 2013 and edited 23 March 2018

Dependencies:
treemix (https://code.google.com/p/treemix/)
sumtrees package in dendropy (http://pythonhosted.org/DendroPy/scripts/sumtrees.html)

Usage:       python treemix_tree_with_bootstraps.py input_file
number_of_migrations_allowed outgroup_name bootstrap_output_directory
                    number_of_bootstraps_to_run size_of_bootstrap_blocks

Make sure Treemix is in your path. If, when installing treemix, there is an issue
locating boost, try using CPPFLAGS to specify the location of the boost libraries
(replacing "PATH_TO_BOOST" with the correct location):
>./configure CPPFLAGS=-I/PATH_TO_BOOST/boost_1_53_0/

"""
import os
import sys
import argparse
from Bio import AlignIO

def get_args():
       parser = argparse.ArgumentParser(
                    description="""Program description""")
       parser.add_argument(
                    "in_file",
                    type=str,
                    help="""The input nexus file"""
             )
       parser.add_argument(
                    "migration",
                    type=int,
                    help="""The number of migration events allowed"""
             )
       parser.add_argument(
                    "outgroup",
                    type=str,
                    help="""The name of the outgroup"""
             )
       parser.add_argument(
                    "out_dir",
                    type=str,
                    help="""The output directory"""
                    )
       parser.add_argument(
                    "bootreps",
                    type=int,
                    help="""The number of bootstrap replicates to run"""
             )
       parser.add_argument(
                    "block_size",
                    type=int,
                    help="""The number of SNPs to include in bootstrap blocks"""
             )
```

```
  return parser.parse_args(
def run_bootreps(in_file, migration, outgroup, out_dir, block_size, bootreps):
       infile = "{0}.gz".format(in_file) # SNP data in treemix format
       reps2 = bootreps
       for i in range(reps2):
              print "\nBootRep: {0}\n".format(i+1)
              outfile = "{0}bootstraps/treemix_bootrep_{1}".format(out_dir, i+1)
              os.system("treemix -i {0} -m {1} -bootstrap -root {2} -o {3} -k {4}
".format(infile, migration, outgroup, outfile, block_size))

def combine_bootreps(out_dir, bootreps):
       outfile = open("{0}cat_trees.tre".format(out_dir), 'wb')
       for i in range(bootreps):
              infile =
open("{0}bootstraps/treemix_bootrep_{1}.treeout.gz".format(out_dir, i+1), 'r')
              for line in infile:
                     outfile.write(line)
              infile.close()
       outfile.close()

def main():
       args = get_args()
       os.system("gzip {0}".format(args.in_file)) # gzip input file
       os.system("treemix -i {0}.gz -m {1} -o {2}_stem".format(args.in_file,
args.migration, args.out_dir)) # build ML tree
       os.system("mkdir {0}bootstraps".format(args.out_dir)) # make dir for bootreps
       run_bootreps(args.in_file, args.migration, args.outgroup, args.out_dir,
args.block_size, args.bootreps) # run bootreps
       combine_bootreps(args.out_dir, args.bootreps) # combine bootreps into one file
       os.system("zcat *stem.treeout.gz | head -1 > out_stem.treeout.tre") #unzip and
remove migration from main ML tree
  os.system("zcat *cat_trees.tre | egrep -v "NA NA NA" > cat_trees.tre") #add boot trees
without migration to concat of trees
       os.system("sumtrees.py --force-unrooted -t ./out_stem.treeout.tre -o
./boottree.tre ./cat_trees.tre") #use sumtrees of Dendropy biopython package
       os.system("head -1 boottree.tre > final.treeout") # use sumtrees as final newick
tree
       os.system("zcat *stem.treeout.gz | head -4 >> final.treeout") #add migration
lines from the original treeout
       os.system("gzip final.treeout") # zip final.treeout

"""
The resulting tree is in NEXUS by default, and can be visualized in e.g. FigTree. You
can also visualize the tree in R using the commands (replacing "PATH_TO_TREEMIX" with
the correct path):
>source("/PATH_TO_TREEMIX/treemix-1.11/src/plotting_funcs.R")
>plot_tree("/PATH_TO_TREEMIX/treemix-1.11/out_stem")
"""

if __name__ == '__main__':
 main()
```