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Genetic Diversity among Chrysicthes Nigrodigitatus from Fresh and Brackish Waterbodies in Nigeria

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Abstract

The research was to determine the morphometric characters and molecular characteristics of Chrysicthes nigrodigitatus from brackish and freshwaters in Nigeria. Four locations were selected according to availability of the fish viz Lagos, Kainji, Lokoja and Yola. The fish were identified using standard identification kits. The genetic diversities of C. nigrodigitatus in the four populations based on mitochrondria(Mt)-dloop, Kainji had 10 segregating sites with an average of 4.381 nucleotide differences; Lagos had 19 segregating sites with an average of 7.464 nucleotide differences, Lokoja and Yola had 11 segregating sites each with averages of 5.400 and 3.76 nucleotide differences. There was genetic differentiation between Lagos and Lokoja, they showed strong within-population variation and greater genetic distance between the two. The haplotype network with loops of C nigrodigitatus from the four populations showed a more complex network.

Keywords: Genetic diversity, Freshwater, Brackish water, *Chrysicthyes nigrodigitatus*

Introduction: Genetic variation in a species enhances the capability of an organism to adapt to the changing environment and is necessary for the survival of the species. This arises between individuals leading to differentiation at the level of population, species and generic groups. The genetic diversity data has varied applications in research on evolution, conservation and management of natural resources including genetic improvement programmes. Molecular genetic markers have a powerful ability to detect genetic structures and uniqueness of individuals, populations or species (Doveri *et al.,* 2008). These molecular markers combined with new statistical developments have revolutionized the analytical power, necessary to explore genetic diversity. Various molecular markers, protein or DNA (mt-DNA or nuclear DNA) are now being used in fisheries management and aquaculture (Duwal *et al.,* 2016). Recently, these markers provide various scientific observations which have importance in aquaculture practice such as: species identification, genetic variation and population structure study in natural populations, comparison between wild and hatchery populations, assessment of demographic bottlenecks in natural populations and propagation-assisted rehabilitation programmes (Chauhan and Rajiv, 2010).

The use of molecular markers such as mitochondria DNA mtDNA, random amplification of polymorphic DNA, and microsatellite DNA has greatly facilitated studies on the genetic structure, diversity, and evolutionary divergence of different fish populations including *Chrysichthys* spp. (Nwafili and Gao, 2016). The use of mtDNA has a great advantage over Nuclear DNA because of its intrinsic ability to resist degradation and its high copy number inside the cell as compared to nuclear DNA (nDNA). Each cell contains around 1000 mitochondria, and there are 2–10 copies of the mtDNA per mitochondrion. The mtDNA D-loop is the only non-coding segment that exists in

the vertebrate mitochondrial genome (Zardoya and Meyer, 1997). This D-loop segment is the most variable part of the mtDNA, and evolves three to five times faster than the rest of the mitochondrial genome (Ferraira *et al*., 2024). Therefore, it is widely used as a genetic marker to assess the origin, phylogenesis, and intraspecific genetic differentiation of animal species (Rasmussen and Morrissey, 2008)* including *C. nigrodigitatus*. In Nigeria, molecular marker based on PCR techniques has been used to determine the population structure and genetic diversity of some species (Ahmad *et al*., 2012; Mojekwu *et al*., 2012) though an emerging area in fisheries management. The objectives of this study are to evaluate the genetic diversity among *C. nigrodigitatus* within a population and between four populations and assess the population structure and genetic differentiation among the four populations,

Materials and Methods: Study Area: The Sampling locations were namely Fakun, Kainji, (Freshwater) and coded as KJ (Figure 1). It is located north of Jebba Lake and a famous fishing village around Kainji Lake. It is a small fishing community that had no light in this settlement of migrant fishers. Tall towers conveying electricity out of Kainji dam pass by their village to supply power to some distant towns and cities within the country, and the Lake itself lies about a thousand metres away. From a distance one can hear the waters roar as well as the hum of powerful machines within. Inside the Lake there is the sound of surging waters. In the village can be heard another different sound of small cheap generators which bring a ray of life and maybe light to this silent place teeming with fishers drawn from many parts of Nigeria.

Epe Lagoon, Lagos (Brackish Water) coded as LA (Figure 2), Epe is a town and a Local Government Area (LGA) in Lagos State, Nigeria, located on the north side of the Lekki Lagoon. It is a Yoruba town located next to the Lagos lagoon with 294 rural and 24 semi-urban communities. Epe is known for its fish market which feeds off the hard work of those men and women whose lives depend on the lagoon – and the fish inside it. Lokoja, River Benue and River Niger confluence (Freshwater) LK (Figure 3). The city serves as a confluence for the two most prominent rivers in Nigeria; River Niger and River Benue. The ancient city of Lokoja has a long tradition of a very active domestic fishing industry and with the rivers Niger and Benue making a confluence at Lokoja makes the city a natural attraction for some artisanal fish farmers who travel many hundreds of miles during dry seasons from Kano and Sokoto to Lokoja where they fish for a few months until the rain begins. Yola, River Benue (Freshwater) YL (Figure 4). It is the capital of Adamawa State and also the seat of Lamido Fombina. It experiences migratory fishers from all over the nation especially the Northern Nigeria. Table 1 is showing the coordinates of the foue locations in Nigeria. The different locations have variations in ecological conditions such as rainfall, temperature, vegetation, etc.

Table 1: Coordinates of Sample Collection Locations.

LOCATION	LATITUDE	LONGITUDE	STATE
Kainji	09°09'53.05"N	04°53'57.01"E	Niger
Lagos (Epe)	06° 35' 02.83" N	03° 59' 00.10" E	Lagos
Lokoja	07° 47' 48.77" N	06° 44' 25.73" E	Kogi
Yola	09° 17' 14.07"N	12° 27' 51.90" E	Adamawa

Extraction of Genomic DNA: The DNA extraction was performed by ACUTIG Laboratories in Abeokuta, Ogun State, Nigeria. The procedure was in the following order: To each Proteinase K (20 mg) tube, 1,040 µl Proteinase K Storage Buffer was added to give a final concentration of ~ 20mg/l. It was stored at -20ºC after mixing.To a tissue sample (≤25 mg) in a microcentrifuge tube, the following solutions were added: 95 µl Distilled Water, 95 µl Solid Tissue Buffer (Blue in colour), 10 µl Proteinase K.The mixture was vortexed for 15 seconds and then incubated in the tubes at 55ºC for at least 3 hours to ensure the tissue is solubilized. In order to remove the soluble debris, the tubes were centrifuged at ≥1200 x *g* for 60 seconds and the

aqueous supernatant was transferred to a microcentrifuge tube. Two volumes of Genomic Binding Buffer (approximately 400 µl) to 200 µl of the supernatant. It was vortexed for 15 seconds.The mixture was transferred to Zyma-Spin™ Column in a new Collection Tube. It was centrifuged at ≥1200 x *g* for 60 seconds and the Collection Tube was discarded with the flow through.

DNA Determination through Electrophoresis: Agarose gel was prepared by dissolving and heating a solution containing 0.3 g of agarose powder in 30 ml of1 x TBE Buffer (Tris, Boric acid, and EDTA). It was incubated in an oven until a fully clear liquid was obtained.8 DNA samples were randomly selected from the total of 40 DNA samples extracted.Mini-One electrophoresis chamber has 9 wells (comb fingers).The clear solution of Agarose was added to the Mini-One electrophoresis chamber with the comb fingers in place. This was covered to protect it from light until it solidified.It was allowed to set and the comb fingers were carefully removed to create wells.5 µl of loading dye was added to 5 µl of DNA sample. It was thoroughly mixed and transferred into the wells.

The Polymerase Chain Reaction.: The materials used included the forward and reverse primer sequences known as AfricanCatfish_Fwd and AfricanCatfish_Rv (Table 2). Other materials were:

i. Biometra Gradient Thermocycler (Gottingen, Germany)
ii. Master Mix ,
iii. DNA Surf Hot Taq Polymerase (10 U/µl) (Stab Vida, Lisbon, Portugal)
iv. Magnetic Beads

Table 2: Primers Sequences Used for the Amplification

Name of Primer	Primer Sequence	Properties
	CATCCTCACCTGAATCGG	52.47°C
African CatFish _Rev	GGG TGCTTGCTAATGAAG	53.78°C

Data Analysis: Genetic diversity indices, such as observed heterozygosity, expected heterozygosity, and F-statistics, will be calculated using software like Arlequin (Excoffier *et al.*, 2005). Analysis of molecular variance (AMOVA) will be conducted to determine the genetic differentiation among the populations.

Results: Genetic Diversities of *C. nigrodigitatus* in Four Populations: Diversity indices of *C. nigrodigitatus* from the four populations based on Mt-dloop (mitochondrial DNA control region) analysis is presented in Table 10. Kainji recorded 10 segregating sites, 5 haplotypes, moderate haplotype diversity (0.905), low nucleotide diversity (0.007), and an average of 4.381 nucleotide differences. Lagos had 19 segregating sites, 7 haplotypes, high haplotype diversity (0.964), moderate nucleotide diversity (0.011), and an average of 7.464 nucleotide differences. Lokoja on the other hand recorded 11 segregating sites, 5 haplotypes, high haplotype diversity (1.000), low nucleotide diversity (0.008), and an average of 5.400 nucleotide differences, and Yola had 11 segregating sites, 7 haplotypes, high haplotype diversity (0.964), low nucleotide diversity (0.006), and an average of 3.786 nucleotide differences. Table 10 showed that the Lagos and Yola populations have higher genetic diversity compared to the Lokoja and Kainji populations.

Table 3: **Diversity Indices of *C. nigrodigitatus* from four Populations based on Mt-dloop**

	Population			
Diversity Indices	**Kainji**	**Lagos**	**Lokoja**	**Yola**
Segregating site S	10	19	11	11
Number of haplotypes H	5	7	5	7
Haplotype diversity Hd	0.905	0.964	1.000	0.964
Nucleotide diversity π	0.007	0.011	0.008	0.006
Average number of nucleotide differences K	4.381	7.46	5.400	3.786

Demographic History of *C. nigrodigitatus* in the Four Locations: The result in Table 13 showed the deviation from neutrality of *C. nigrodigitatus* in the four populations. Kainji had positive Tajima's D (0.394), positive Fu's Fs (0.039), Fu and Li's D (0.248) and F (0.283) are all positive. Lagos had a Tajima's D (0.097), negative Fu's Fs (-1.031), Fu and Li's D (0.071) and F (0.078) close to zero. Lokoja on the other hand a negative Tajima's D (-0.452), negative Fu's Fs (-1.223), negative Fu and Li's D (-0.298) and F (-0.298). Yola recorded a negative Tajima's D (-0.538), strongly negative Fu's Fs (-2.603), Fu and Li's D (-0.360) and F (-0.445) were also negative.

Table 4: Deviation from Neutrality of *C. nigrodigitatus* from Four Populations based on mt-dloop

	Population			
Statistical test	**Kainji**	**Lagos**	**Lokoja**	**Yola**
Tajima's D	0.394	0.097	-0.452	-0.538
Fu's Fs	0.039	-1.031	-1.223	-2.603
Fu and Li's D	0.248	0.071	-0.298	-0.360
Fu and Li's F	0.283	0.078	-0.298	-0.445

Population Structure and Genetic Differentiation of the Four Populations: In the result shown in Table 2, within population variation was observed to be 79.98% and among population variation was 20.02%. Pairwise F_{ST} was 0.20019 which was a low index. Figures 1and 2 are heatmaps presenting a combination of different metrics. The heatmaps representing pairwise F_{ST} values among the four populations of Kainji, Lagos, Lokoja, and Yola. Darker blue areas represent higher F_{ST} while lighter blue to white areas represent lower F_{ST}. Kainji population has low differentiation with compared to Yola and Lokoja population (indicated by very light colours), but had higher differentiation with Lagos (indicated by the darker color) population. Lagos showed high differentiation with all the other populations, especially with Lokoja and Yola population. Lokoja and Yola seem relatively similar to each other (lighter shades), indicating lower differentiation.

The **between populations are green, within populations orange-red, and Nei's distance blue. Green Scale (Between Populations):** This indicated genetic differentiation between the populations, similar to F_{ST} but with a focus on a specific measure of differentiation. **Darker green** indicated higher differentiation between the populations, while **lighter green** or **white** indicated lower differentiation. For example, the **Kainji-Lagos** pair showed darker green, indicating high differentiation between these two populations. **Orange-Red Scale (Within Populations):** This showed genetic variation within the same population. The **darker orange or red** the colour, the higher the genetic diversity within that population. The **Lagos** and **Lokoja** populations showed strong within-population variation (indicated by darker orange and red), meaning there was significant genetic diversity within these populations. **Blue Scale (Nei's Distance):** Nei's genetic distance measures the genetic divergence between populations. **Darker blue** indicated greater genetic distance. The **Lokoja-Lagos** and **Lokoja-Yola** pairs showed darker blue, indicating greater genetic distance between these pairs.

*A haplotype network (Figure 9) illustrated the relationships among haplotypes, showing how they are connected through mutational steps. There were 22 haplotypes from Lagos, Lokoja, Kainji, and Yola. Each haplotype was indicated by a specific colour as indicated in the figure. The central haplotype (Hap 11, Hap 20) which is the most common haplotype that is connected to many others (7 connections each),

indicated an ancestral or widespread haplotype. The peripheral haplotypes are mostly fewer common haplotypes which are connected to the central haplotype or other peripheral haplotypes and indicated derived or localized haplotypes. The connections lines or branches connecting haplotypes represented mutational steps (single nucleotide changes), while loops or reticulations indicated recurrent mutations or recombination events.

Table 5: Analysis of Molecular Variance of the Four Populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}
Among populations	3	112.725	3.20136	Va 20.02	0.20019
Within populations	27	345.339	12.79034	Vb 79.98	
Total	30	458.065	15.99171		

Discussion: Genetic Diversity of *C. nigrodigitatus* in the Four Populations: Studies of population genetic diversity and molecular evolution can provide useful information necessary for species conservation and population management (Okonko *et al*., 2022). The capability of the fingerlings, juveniles and adults to undertake migrations could contribute to shaping the genetic diversity and population structure (Nwafili and Gao, 2016). Chances of survival of individuals and evolutionary ability of a population rely on maintenance of genetic diversity (Hoglund, 2009; Sahoo *et al*., 2020). African Agenda 2063 encompasses in its main aims the sixth goal, which is concerned by blue economy development and promotion of sustainable use of aquatic resources (Hamed-Mohamed *et al*., 2023). This has made this study of the genetic diversity of *C. nigrodigitatus* in four locations in Nigeria timely. This is a pilot study comparing the four populations of the species using Mitochondria displacement loop (mtDNA d-loop). The four population presented a high haplotype diversity which is an attribute to long evolutionary history in a large stable population. Nwafili *et al*. (2013) reported a similar result on *C. nigrodigitatus* and *C. walkeri* from the Lagos Lagoon. The report of Sita *et al*. (2018) on *Chrysichthys* species in Bia River, Cote D'ivoire also agreed with this report. The Segregating sites presented the positions in the DNA sequence where there were variation among individuals within the four populations. Lagos population had the highest number of segregating sites (19), suggesting greater genetic variation at individual nucleotide positions. Kainji had the lowest (10), indicating comparatively less variation. The higher number of segregating sites presented by Lagos population is an indication that it might have a higher mutation rate or more genetic mixing compared to the other populations, possibly due to a larger population size or greater gene flow. Recognising genetic diversity is an essential part of biodiversity conservation, enabling populations and species to survive and evolve in accordance with environmental changes (Gu *et al*., 2021). Molecular markers (mitochondrial and microsatellites DNA) are commonly used to analyse genetic diversity, which is of great significance to the sustainable use of biodiversity and biological resources (Gu *et al*., 2021). A haplotype is a group of alleles that are inherited together. The number of haplotypes (H) reflects the genetic diversity within a population. Lagos and Yola each had 7 haplotypes, indicating more diverse haplotypes compared to Kainji and Lokoja, which each had 5. It is believed that higher haplotype count in Lagos and Yola suggested that the populations may have higher genetic diversity and potentially more evolutionary adaptability. Diversity of the Haplotype (Hd) is the probability that two randomly chosen haplotypes from the population

are different. Lokoja gave the highest possible haplotype diversity (1.000), indicating all individuals in this population had unique haplotypes. Kainji had a slightly lower haplotype diversity (0.905), showing less diversity in haplotype distribution. Higher haplotype diversity in Lokoja, Lagos, and Yola points to these populations being genetically well-differentiated, while Kainji showed less variation, potentially from a more isolated or smaller population. Sahoo *et al*. (2018) opined that higher number of private haplotypes might be due to independent origin of haplotypes through mutation and could be used as population specific marker for stock identification. The haplotype diversity and nucleotide diversity for *C. nigrodigitatus* populations in this study were Lokoja (1.000) and Lagos (0.011), respectively, that showed high haplotype and nucleotide diversities. This result does not agree with the report of Sahoo *et al*. (2020) who studied Indian Catfish using mtDNA d-loop and obtained moderate haplotype and low nucleotide diversities of 0.652 and 0.00129 respectively. Similar level of genetic diversity was reported earlier for *Chrysicthys nigrodigitatus* populations from Cross River (Adelieje *et al.*,2020). The observed Hd in the present study was above the range observed in other freshwater fishes (Habib *et al*., 2012, Hd=0.876). Nucleotide diversity (π) is the average difference per nucleotide site between two DNA sequences in a population. In this report Lagos had the highest nucleotide diversity (0.011) and the least was Yola (0.006). The higher nucleotide diversity in Lagos suggested that it may have accumulated more mutations or has experienced more gene flow than the other populations. Yola's lower diversity may suggest either a smaller population size or more recent bottleneck effects. Hamed-Mohamed *et al*. (2023) reported a similar case for the genus *Chrysichthys* in Egypt that high haplotype diversity corresponded to low nucleotide diversity. This indicated low flow of genetic information and little divergence among populations (De Jong *et al*., 2011). The Low nucleotide diversity observed here might be due to founder effect from different colonization events as well as anthropogenic activities (Adelieje *et al.*,2020). High/moderate haplotype diversity and low nucleotide diversity as evidenced in magur populations might be due to the rapid expansion and population growth after a period of low effective population leading to the retention of new mutations (Avise *et al*., 1984; Rogers and Harpending, 1992). It is a well-known fact that large population size could maintain high haplotype diversity within a population (Nei, 1987). It could now be envisaged that the four populations studied have large sizes since all of them had high haplotype diversity. However, populations with higher haplotype and nucleotide diversity, like Lagos and Lokoja, are likely better equipped to adapt to environmental changes, whereas Kainji and Yola may have less adaptability

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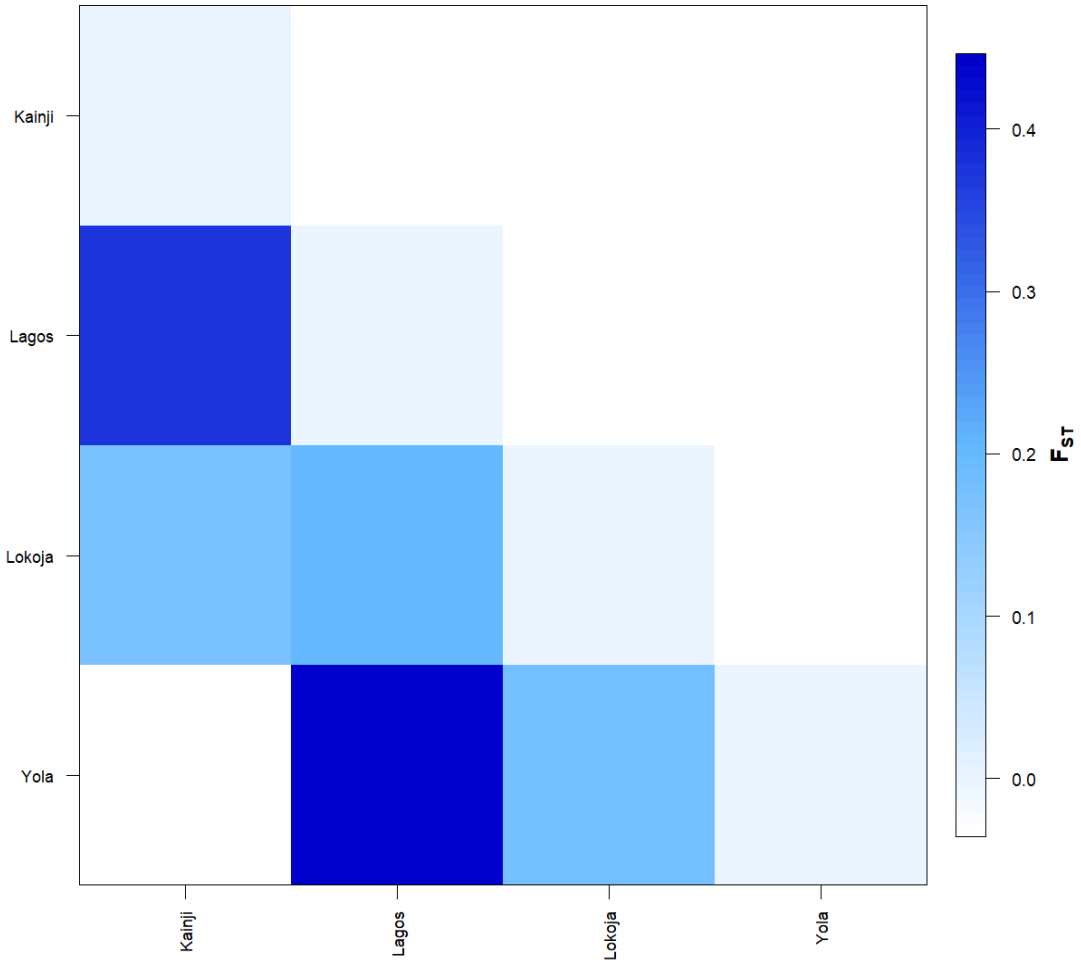

Figure 1: A Heatmap Pairwise Fixation Index Matrix for the Four Populations

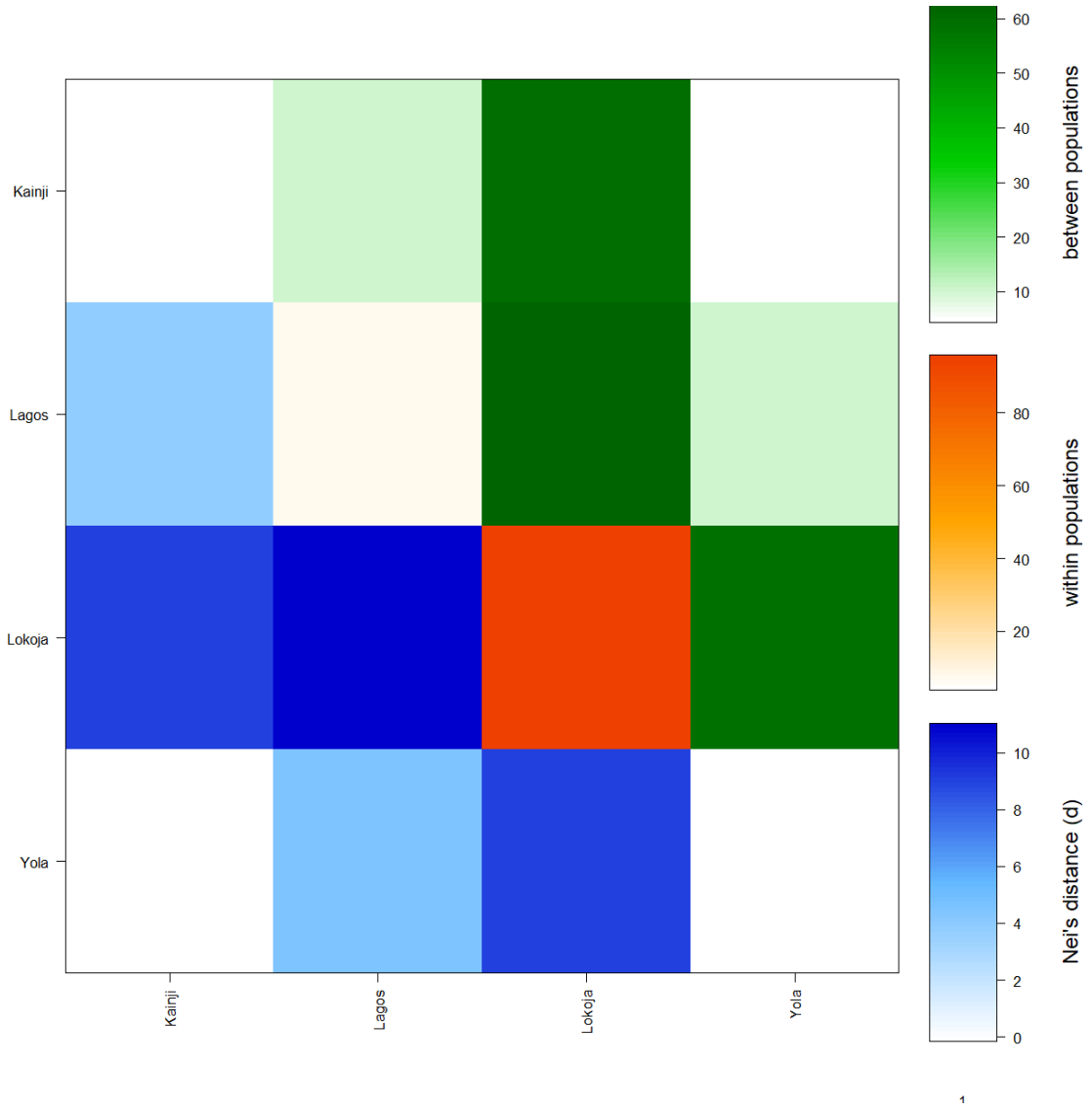

Figure 2: A Heatmap showing Average Number of Pairwise Differences

Conclusion: Populations with higher genetic variation, such as those in Lagos and Lokoja, may be better suited to cope with environmental changes, while Kainji and Yola might face reduced adaptability. These findings align with global patterns in fish genetics, emphasizing the importance of integrating genetic data into sustainable fisheries management and conservation efforts in alignment with Sustainable development goals. The F_{ST} value (0.20019) indicated moderate genetic differentiation, suggesting limited dispersal and gene flow between populations. These results emphasized the need for location-specific conservation efforts to maintain genetic diversity and ensure the sustainability of fish populations towards food security.

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