Genetic Relationship between the Different Ethno-Linguistic Groups of Mezam and Momo Divisions of the North West Region of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author NPF performed the experiments, was involved in conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, software visualization writing-original draft writing-review & editing. Author FCN was involved in conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, validation, writing-review & editing. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Although the many ethnolinguistic groups of the Mezam and Momo Divisions of the North West Region of Cameroon are historically known to be related, no genetic study has hider to be conducted to verify the claim. This study was therefore aimed at determining the genetic relationship between the different ethno-linguistic groups of Mezam and Momo divisions of north west Cameroon using 6 Y-STR loci.

Materials and methods: Venous blood samples were collected from, at least 30 consented participants from each of 11 ethnolinguistic groups previously identified in the study area. Genomic DNA was isolated and six Y-Chromosome Short Tandem Repeats (Y-STRs): DYS388, DYS389I, DYS390, DYS391, DYS392, and DYS393, were genotyped using standard techniques and the phylogenetic relationship between these groups established.
1. INTRODUCTION

Cameroon is a linguistically diverse country with over 247 indigenous languages [1] which belong to 3 established language families: The Afro-Asiatic, the Nilo-Saharan and the Niger-Congo families. The Niger Congo languages also consist of a Senegambian language (Fulfule), Adamawa and Benue-Congo languages. The languages belonging to the Nilo-Saharan and Afro-Asiatic families are spoken in the northern part of the country, while Niger-Congo languages, which are the most highly represented in Cameroon, are spoken in the southern parts of the country [1]. Momo and Mezam Divisions of the north West Region of Cameroon are home to about 17 Niger Congo languages which results in the many ethnolinguistic groups of the region. Although these groups are historically known to be related, there exist no genetic study to establish the claim.

The importance of population genetic relationship studies cannot be over emphasized; tracing the genetic origin of a population; achieving more complete identification of missing persons or disaster victims [2]; determination of populations at risk of certain gene related diseases [3] and pharmacogenetics [4].

Genetic markers located on the Y-chromosome are commonly used in genetic genealogy to establish genetic relatedness among populations because they combine genetic data and family history [5]. The Y-chromosome is one of the smallest in the human genome and represents about two to three percent of the haploid genome [6]. It is different from the other chromosomes in that: it is not essential for the life of an individual as females survive without it, half of it consists of tandemly repeated satellite DNA and the rest carries few genes, and most of it does not recombine. It is therefore passed unchanged from father to son. However, these properties make the Y chromosome a better tool for investigating recent human evolution from a male perspective [7].

As a result of the uniparental inheritance pattern of the Y-chromosome, Y-chromosome STRs are a powerful tool in molecular forensics [8] where they are very useful in paternity testing especially in cases where the biological father of a male child is unavailable for testing, but at least one male relative of the father is available. Y-STRs can also be used in solving cases involving male/female mixtures such as in rape cases [9] where it could be used to determine exclusively the male fraction of the DNA, belonging to the perpetrator. In genetic genealogy, Y-STRs are used in tracing members of a patriline.

Linguistic and genetic patterns are similar in that, they result from biological and social interactions of individuals in the society hence, there is a reason to expect the coevolution of linguistics and genetics [10,11]. Nonetheless, different processes affect genetics and linguistics which tends to reduce a strict correspondence between them. Genetic transmission is vertical from parents to offspring and therefore, the only forces that diversify the genetics of a population is segregation and recombination. On the other hand, language transmission can be vertical or horizontal [10], when two linguistic groups merge, one language is usually suppressed but when two genetically different populations merge, the result is admixture which gives rise to greater genetic diversity. This Therefore implies that, population groups speaking same (closely related) or completely different languages may share the same/different genetic ancestor [12]. This study was therefore aimed at determining the genetic relationship between the different ethnolinguistic groups of Mezam and Momo Divisions of the North West Region of Cameroon as classified by Ethnologue, 20th edition [13] using Y-STRs.
2. MATERIALS AND METHODS

Eleven populations including Awing, Pinyin, Bafut, Mendankwe-Nkwen, Bambili-Bambui, and Bali-Nyonga of Mezam Division and Batibo, Meta, Ngie, Oshie, Bifang, of Momo Division were studied. Six Y chromosome STRs; DYS388, DYS389I, DYS390, DYS391, DYS392 and DYS393 were genotyped in an attempt to determine the genetic relatedness among them. Also, existing data of ten additional Cameroonian populations, (Bakaka, Bassa, Fulbe, Fali, Tuouri, Mandara, Udeme, Podokwo, Ewondo and Bamileke) previously studied [14,15] were included in the analysis of interpopulation relationship. Data for Africa, Europe and Asia in a study by Rustamov [16] were included as well.

Administrative authorization to carry out research in Momo and Mezam Divisions was obtained from the North West Regional Delegation of Public Health, Cameroon (Reference N° 83/ATT/NWR/RDPH). Sampling was random and only males of at least 21 years old whose paternal grandfathers originated from the 11 linguistic groups of Momo or Mezam Division took part in the study. About one millilitre of venous blood was collected by venepuncture into EDTA tubes from at least 30 consented persons from each linguistic group.

Genomic DNA was isolated from frozen whole blood samples (samples stored at -20°C for four to five months) using the method of Iranpur and Esmailizadeh [17] and separated on one percent agarose gel to confirm the presence of DNA and to check the quality of the extracted samples. Six Y-STR polymorphisms (DYS388, DYS389I, DYS390, DYS391, DYS392, and DYS393) were analysed. The primers used in this study were obtained from the Y-STR fact sheet [18] and are shown on Table 1.

DNA amplification was carried out using the OneTaq 2X PCR Reaction Mix (New England Biolabs). Each 25 µL PCR reaction tube contained 12.5 µL of OneTaq 2X PCR Reaction Mix, 0.5 µL (10 µM) of forward and reverse primers, one microlitre (sterile water for the PCR control tube) of template DNA (50 ng) and 10.5 µL of sterile water to make up the volume to 25 µL. The PCR amplification conditions were 95°C for two minutes followed by 35 cycles of one minute at 94°C, one minute at 55°C, and two minutes at 68°C. The reactions ended with 10 minutes incubation at 68°C. A Peltier based PCR thermal cycler was used for all amplification reactions. The PCR amplicons were then separated on a six percent urea denaturing polyacrylamide gel. The gel was silver-stained according to the method of Benbouza and colleagues [19]. Samples whose bands appeared at the same position on the gel were grouped together and five, representative of each group were sequenced (Genewiz sanger sequencing, Germany) to determine the number of STR repeats in each sample. The number of repeat units were determined from the sequencing trace file by direct counting. Allele/haplotype frequencies were determined using the gene counting method and the power of discrimination/exclusion for each Y chromosome marker was represented by its gene diversity (h):

| SN | locus | Primer Name | Repeat Unit | Primer Sequence (5’ →3’) |
|----|-------|-------------|-------------|--------------------------|
| 1  | DYS388 | DYS388F | ATT         | GTGAGTTAGCCGTTCAGGA     |
|    |       | DYS388R | AAT         | CAGATCGCAACACTGCG        |
| 2  | DYS389I | DYS389IF | (TCTG)n(TCTA)m(TCTG)p(TCTG)q | CCACTCTCATCTGTATTATCTATG |
|    |       | DYS389IR | (CAGA)q(CAGA)p(TAGA)m(CAGA)n | TCTTATCTCCACCCACAGA |
| 3  | DYS390 | DYS390F | (TCTA)m(TCTG)n | TATATTTACACTTTTTGGCC |
|    |       | DYS390R | (CAGA)n(TAGA)m | TGACGTAAATAGAAACATTTGC |
| 4  | DYS391 | DYS391F | TCTA         | CATTTCAATCATACACCCA     |
|    |       | DYS391R | TAGA         | GATTTCTGTGTTGCTG        |
| 9  | DYS392 | DYS392F | TAT          | TCATTAATCTAGCTTTTTAAAAACAA |
| 10 | DYS392 | DYS392R | ATA          | AGACCCAGTGATGCAATGT     |
| 11 | DYS393 | DYS393F | AGAT         | GTGTCTTTCTACTTGTCATAC    |
| 12 | DYS393 | DYS393R | ATCT         | AACTCAAGTCCAAAAATGAGG   |

F = forward primer, R= Reverse primer
h = 1 − Σx_i^2 ...................................... (1)

where x represents the frequency of the i^{th} allele

[20] The number of haplotypes (H) were also
determined by the gene counting method and
haplotype diversity (D) determined using the
formula:

D = (n/(n-1) 1 − Σx_i^2) ....................... (2)

where x_i is the frequency of the i^{th} haplotype. Y
haplotypes were defined by the combination of
tested alleles at each locus. The allele frequency
data obtained in this study and those obtained
from other studies [15,16,14] in other
Cameroonian populations, Africa, Europe and
Asia were used to generate a Neighbor joining
[21] phylogenetic tree in Poptree2 [22]. The
number of bootstrap replications was set at 1000.
The tree was drawn to scale with the branch
lengths measuring the number of differences
between haplotypes.

3. RESULTS

Upon electrophoresis, the alleles of each locus
analysed was determined from the gels. Fig. 1
represent the gel pictures indicating the number
of alleles obtained for each locus after
electrophoresis and silver staining of the PCR
amplicons.

Two amplified products were obtained for locus
DYS388 and DYS393 while the other four alleles
had a single product.

![Gel Images]

Fig. 1. Resolution of 6 Y-chromosome STRs on 6% urea denaturing polyacrylamide gel
### Table 2. Allele frequency of each locus per linguistic group

| Locus   | Allele | Awing | Bali | Mendankweh | Bambili | Pinyin | Bafut | Batibo | Meta | Bifang | Oshie | Ngie |
|---------|--------|-------|------|------------|---------|--------|-------|--------|------|--------|-------|------|
| DYS388  |        |       | 1    | 1          | 1       | 1      | 0.93  | 1      | 1    | 1      | 1     | 1    |
|         | 12     | 0     | 1    | 1          | 1       | 1      | 0.0625| 1      | 0    | 0      | 0     | 0    |
| DYS389I |        |       | 1    | 1          | 1       | 1      | 1     | 1      | 1    | 1      | 1     | 1    |
| DYS390  | 21     | 1     | 1    | 1          | 1       | 1      | 1     | 1      | 1    | 1      | 1     | 1    |
| DYS391  | 10     | 1     | 1    | 1          | 1       | 1      | 1     | 1      | 1    | 1      | 1     | 1    |
| DYS392  | 11     | 1     | 1    | 1          | 1       | 1      | 1     | 1      | 1    | 1      | 1     | 1    |
| DYS393  | 8      | 0.2286| 0    | 0.1563     | 0.2777  | 0.2333 | 0.5   | 0.3666 | 0.2  | 0      | 0.0666| 0.0416|
|         | 9      | 0.7714| 1    | 0.8438     | 0.7222  | 0.7667 | 0.5   | 0.6333 | 0.8  | 1      | 0.9333| 0.9583|

### Table 3. Haplotypes observed and their frequencies in each linguistic group

| Haplotype | DYS:388,389a,390, 392,393,391 | Awing | Ngie | Bali | Mendankwe | Batibo | Meta | Oshie | Bifang | Bambili | Pinyin | Bafut |
|-----------|-------------------------------|-------|------|------|------------|--------|------|-------|--------|---------|--------|-------|
| I         | 12,13,21,11,9,10              | 0     | 0    | 0    | 2          | 0      | 0    | 0     | 0      | 0       | 0      | 0     |
| II        | 11,13,21,11,9,10              | 27    | 23   | 28   | 24         | 18     | 24   | 28    | 30     | 26      | 23     | 15    |
| III       | 12,13,21,11,8,10              | 0     | 0    | 0    | 1          | 1      | 0    | 0     | 0      | 0       | 0      | 0     |
| IV        | 11,13,21,11,8,10              | 8     | 1    | 0    | 5          | 11     | 6    | 2     | 0      | 10      | 7      | 15    |
| total     | 35                             | 24    | 28   | 32   | 30         | 30     | 30   | 30    | 36     | 30      | 30     | 30    |
Four (DYS389I, DYS390, DYS391 and DYS392) of the six STRs analysed in this study had an allele frequency of one in all 11 linguistic groups. DYS393 had two different alleles with eight and nine repeats however, the allele with nine repeats was dominant in 10 of the 11 linguistic groups. In the Bafut population, the two alleles were present at equal proportions. Locus DYS388 also had two alleles, 11 and 12 repeats. The allele frequency of the allele with 11 repeats was one in nine of the 11 populations. Table 2 shows the allele frequencies of the different loci typed in this study.

A total of four haplotypes were obtained and haplotype II (11-13-21-11-9-10 corresponding to DYS388-DYS389I-DYS390-DYS392-DYS393- DYS391), was the most abundant haplotype in 10 of the 11 populations under study. Haplotype II and IV were present at equal proportions in the Bafut linguistic group. In the populations of Bali-Nyonga and Bifang, only haplotype II was present. Haplotypes were found in Batibo and Mendanwe-Nkwen and occurred just once. Table 3 shows the different haplotypes observed and their frequencies in the different study populations.

A genetic and linguistic relationship was observed in this study as all 11 linguistic groups with languages belonging to the same language family (Bantoid) formed a cluster and a common origin with the Bakaka population. Interpopulation relationship is demonstrated on the phylogenetic tree (Fig. 2).

Tree constructed from allele frequency data with bootstrap 1000. The distance scale represents the number of differences between the haplotypes.
4. DISCUSSION

In this study, six Y-STR loci were successfully genotyped in 11 ethnolinguistic groups of Momo and Mezam Divisions of the North West Region of Cameroon. Four loci (DYS390, DYS391, DYS389I and DYS391) had an allele frequency of 1 in all 11 groups, hence a gene diversity value of zero. The most diverse locus was DYS393 which had two alleles with eight and nine repeats. These STR loci are located on the Y chromosome which does not recombine and therefore, mutation is the only form of diversification [7]. Also, these STRs are known to have a low mutation rate and are termed slowly mutating STRs, with mutation rates between $10^{-4}$ and $10^{-2}$ per locus per generation (Y chromosome STR Haplotype Reference Database, YHRD). However, these STR loci with low mutation rates are preferred in genetic genealogy and evolutionary studies, where they are used to identify closely related male lineages [23]. Males who share a common ancestor should have the same alleles for the different loci as it is the case in this study. Loci with very low gene diversity values are as well not suitable in person identification cases such as in paternity testing and in solving cases of sexual assault since many individuals share the same allele. As a result, the loci typed in this study cannot be employed in person identification in Momo and Mezam Divisions of Cameroon but can be used in cases of exclusions.

The results obtained in this study are similar to those obtained in an African population for locus DYS389I, DYS390, DYS391 and DYS392 which had dominant alleles with 13, 21, 10 and 11 repeats respectively [16]. These were also the dominant alleles in the Bakaka, Bassa, Fulbe [15], Ewondo and Bamileke populations of Cameroon [14] and the European population [16]. However, our results were more homogenous, characterised by very low gene diversity values (<0.5) as opposed to higher gene diversity values ranging from 0.5149 for DYS388 to 0.7874 at locus DYS390 [16]. The dominant allele for locus DYS388 had 11 repeats in this study but had a frequency of 0.02 in the study by Rustamov which had a dominant allele with 13 repeats (frequency of 0.62) among Africans [16]. The two alleles of locus DYS393 observed in this study were absent in other populations.

The samples in this study were collected from males whose paternal grandfathers are indigenes of the different linguistic groups. In other words, samples were collected from distinct population groups, known to have the same ancestral origin. It was therefore expected that all the Y-STR loci typed in each population should have a frequency of 1 and a gene diversity of zero, as well as the same haplotype within each group. A justification for the few samples that had different alleles could be the fact that some of the participants may be uncertain about their origin, others may lie about their origins while some of the grandparents of the tested individuals might be immigrants from other parts of the country. However, the dominant alleles could be considered as the Y-STR genetic identity of the people of the different linguistic groups of Momo and Mezam Divisions of the North West Region of Cameroon.

A total of four haplotypes were observed in all study groups. Haplotype II was the most abundant in 10 of the 11 populations however, in the Bafut population it accounted for 50% of the total number of haplotype while haplotype IV accounted for the remaining 50%. This codominance observed in the Bafut population suggests the admixture of two different populations [24] or a mutation in the locus of the Y-chromosome of the early generations of this population many years ago [8]. Haplotype IV differed from haplotype II only at a single locus (DYS393). Therefore, haplotype II may be considered as the founder haplotype of the remaining 10 linguistic groups of Mezam and Momo Divisions, North West Region of Cameroon. If the Y-STR haplotypes of all other populations within the country are studied, it could be used in tracing the geographic origins of missing persons. Also, the genetic homogeneity observed is consistent with the findings of Coia et al. [14] who observed greater genetic homogeneity in the southern part than in the northern part of Cameroon. This genetic homogeneity correlates with the linguistic homogeneity within these populations. The different languages spoken in the different populations belong to the same linguistic classification (Niger-Congo, Atlantic-Congo, Volta-Congo, Benue-Congo, Bantoid, Southern, Wide Grassfields, Narrow Grassfields) [13].

The NJ phylogenetic tree obtained (Fig. 2) can be divided into two main branches with Africa as the root of the populations of Europe, Asia, Mandara, Podokwo, Tupuri and Uldeime. On the other hand, the populations of Fali, Bassa, Bakaka and the 11 populations in this study
share a common origin with Africa. Among the linguistic groups of Mezam division, Awing and Pinyin are closest in terms of linguistics [13], geographic location (neighboring villages) and genetically (Y chromosome STRs) in this study.

Five of the six linguistic groups of Mezam Division (Awing, Pinyin, Mendankwe-Nkwen, Bambil-Bambui and Bafut) which are linguistically related [13] and have the same linguistic classification (Niger-Congo, Atlantic-Congo, Volta-Congo, Benue-Congo, Bantoid, Southern, Wide Grassfields, Narrow Grassfields, Mbam-Nkam, Ngemba.) are also closely related in this study as they form a cluster and a common origin (Mendankwe-Nkwen).

Three of the five linguistic groups of Momo Division employed in this study form a cluster together with the Bali-Nyonga population however, Bali-Nyonga and Bifang are closer and share the same origin, Ngie. This is in line with the history of the people of Ngie who express that they are one and share a common origin as the Oshie, Meta, Widikum (Bifang), and Batibo/Moghamo peoples [20]. Batibo of Momo division is also closest to Bafut of Mezam Division and belong to the same cluster with Meta which is known to have a linguistic similarity of 47% with Moghamo (Batibo) [24]. Overall, all 11 ethnolinguistic groups employed in this study formed a cluster indicating a common origin with the Bakaka population which is linguistically classified by Ethnologue [13] as (Niger-Congo, Atlantic-Congo, Volta-Congo, Benue-Congo, Bantoid, Southern, Narrow Bantu, Northwest, A, Ewondo-Fang (A.72). This group of people are known to have originated from the Nigerian-Cameroon Plataeu together with the Bassa population [14].

As a preliminary study which makes use of only a few Y-STR loci, more work has to be done to analyse a larger number of Y-STRs, single nucleotide polymorphisms, mitochondrial DNA, and a larger number of populations, in order to have a more concise conclusion on the genetic relationships between different Cameroonian populations.

5. CONCLUSION

In conclusion, the allele frequencies of 6 Y-STR loci have been determined in 11 ethnolinguistic groups of Mezam and Momo Divisions, North West Cameroon. All populations studied share a common root, Bakaka and belong to the Bantoid family of languages, implying a linguistic and genetic relationship.

CONSENT

A written informed consent was obtained from all study participants before samples were collected.

ETHICAL CONSIDERATIONS

An ethical clearance was obtained from the institutional review board, Faculty of Health Sciences, University of Buea Cameroon (Reference:2018/136/UB/SG/IRB/FHS).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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