



(RESEARCH ARTICLE)



GENETIC STRUCTURE OF *SIMULIUM DAMNOSUM* S.L. POPULATIONS IN THREE EPIDEMIOLOGICAL PATTERNS OF ONCHOCERCIASIS IN COTE D'IVOIRE

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ABSTRACT

The *Simulium damnosum* sensu lato complex comprises several species that act as vectors for onchocerciasis, which differ in terms of geographical distribution, ecology, dispersal capacity and vector competence. Understanding their genetic structure is essential for assessing connectivity between populations. The objective of this study was to characterise the genetic structure of *S. damnosum* s.l. populations in three epidemiological regions affected by onchocerciasis in Côte d'Ivoire (Soubré, Bouaflé and Touba), using microsatellite markers. Simuliids were collected between December 2024 and October 2025 using human bait, and were then genotyped using four microsatellite loci. Factor analysis of correspondences highlighted two genetic groups, whilst classification analyses identified three to four genetic groups comprising individuals from the three study sites. The fact that individuals are not grouped according to their geographical origin reflects a low level of genetic differentiation between populations. These results indicate the existence of significant gene flow, which supports the hypothesis of active dispersal of black flies in the areas studied. Such connectivity could facilitate the spread of genetic variants between regions and influence the transmission dynamics of onchocerciasis. This study therefore provides new insights into the genetic organisation of *S. damnosum* s.l. populations in Côte d'Ivoire.

Keywords: Genetic structure, *Simulium damnosum* s.l., Microsatellite markers, Onchocerciasis, Côte d'Ivoire

1 INTRODUCTION

Onchocerciasis, caused by the filarial nematode *Onchocerca volvulus* Leuckart, 1893, is transmitted to humans through the bite of an infected female black fly belonging to the *Simulium damnosum* complex Theobald, 1903. This disease is a major public health problem in several regions of sub-Saharan Africa, where it mainly affects populations living near watercourses that are conducive to the larval development of the vectors ¹. Chronic infection can cause severe skin and eye symptoms, which may lead to irreversible visual impairment ². Despite the progress made in combating onchocerciasis through mass drug administration, the continued transmission of the disease in certain areas remains a major obstacle to its sustainable elimination. The *Simulium damnosum* s.l. complex comprises several species that differ, in particular, in terms of their geographical distribution, ecological requirements, dispersal capabilities and vector efficiency. These differences may influence the dynamics and distribution of *Onchocerca volvulus* transmission and, consequently, the epidemiology of onchocerciasis ³. Indeed, populations of *Simulium* flies may exhibit varying genetic structures depending on their degree of connectivity and dispersal. Migration of adults between breeding sites may promote gene flow and limit genetic differentiation between populations, whilst geographical or ecological isolation may, conversely, promote their divergence⁴. Characterising this genetic structure is therefore essential for understanding the dynamics of vector populations and their potential role in the transmission of onchocerciasis. Molecular markers, particularly microsatellites, are particularly well-suited tools for studying the genetic diversity and structure of vector populations^{5,6}. These markers, which are highly polymorphic, codominant and generally neutral, enable the assessment of intra and inter-population genetic diversity, gene flow, genetic differentiation and the degree of isolation between populations. Their use in black flies thus helps to identify any genetic subdivisions between populations from different transmission areas and to gain a better understanding of the mechanisms underlying vector dispersal ⁴. In Côte d'Ivoire, onchocerciasis remains a disease of epidemiological significance, with transmission varying across regions. This heterogeneity is linked, in particular, to the ecological and hydrographic characteristics of the areas concerned ⁷. Populations of *S. damnosum* s.l. colonise various types of watercourses and may vary in terms of their abundance, distribution and ability to transmit *O. volvulus* ⁸. However, the data available on the genetic structure of *S. damnosum* s.l. populations in the various epidemiological zones of Côte d'Ivoire remain limited. In particular, the question of whether populations belonging to distinct epidemiological facies genetic differentiation remains insufficiently documented. Studying the genetic structure of *S. damnosum* s.l. populations in different epidemiological facies could therefore provide important information on their degree of connectivity, their dispersal potential and the spatial dynamics of onchocerciasis transmission. This information could also help to improve our understanding of the epidemiology of the disease and guide strategies for surveillance and vector control. The overall aim of this study was to analyse the genetic structure of *S. damnosum* s.l. populations from three epidemiological regions of onchocerciasis in Côte d'Ivoire using microsatellite markers, in order to gain a better understanding of their genetic organisation and their dispersal dynamics between these different transmission zones.

2 MATERIALS AND METHODS

2.1 Study area

This study was carried out in three departments of Côte d'Ivoire (Figure 1), selected to cover an ecological gradient representative of the country's main phytogeographical facies. These include, in particular, the Soubré department, situated in the south-west of the country within the dense rainforest zone; the Bouaflé department, situated in the central-west of the country within the forest-to-humid savannah transition zone and the Touba department, situated in the north-west of the country within the humid savannah zone.

2.2 Criteria for selecting study sites

The selection of the three study areas was based on a set of entomological and epidemiological criteria. From a hydrological perspective, each of the selected departments is crossed by a major watercourse which serves as a confirmed larval habitat for species of the *Simulium damnosum* complex ; these include the Sassandra river at Soubré, the Marahoué at Bouaflé and the Bagoé, a tributary of the Sassandra, in Touba. From an epidemiological point of view, these localities correspond to three epidemiological profiles of onchocerciasis, characterised by the presence of populations of *S. damnosum* s.l. and by a level of nuisance caused by simuliids that varies according to location and season ⁹.

2.3 Simulium capture

Simulium flies were collected from December 2024 to October 2025 in three epidemiological facies affected by onchocerciasis in western Côte d'Ivoire : Soubré (south-west), Bouaflé (central-west) and Touba (north-west). At each site, the captures were carried out by a team of two people who had given their informed consent. The two collectors took turns at one-hour intervals to ensure that collection operations continued uninterrupted. Female *S. damnosum* s.l. that came to gorge on the operator were captured individually using haemolysis tubes. As *S. damnosum* s.l. females bite

preferentially on the lower limbs, only the legs of the vector collectors were bared. Each tube containing a captured blackfly was hermetically sealed and labelled with the collection site, date, and time. Collections were carried out daily from 7.00 am to 6.00 pm, for three consecutive days at each site. In the field, the tubes containing the blackflies were stored in a portable cooler between two layers of absorbent cotton wool and subsequently transported to the laboratory for further analysis. A minimum sample size of 600 females per site was set as the collection target.

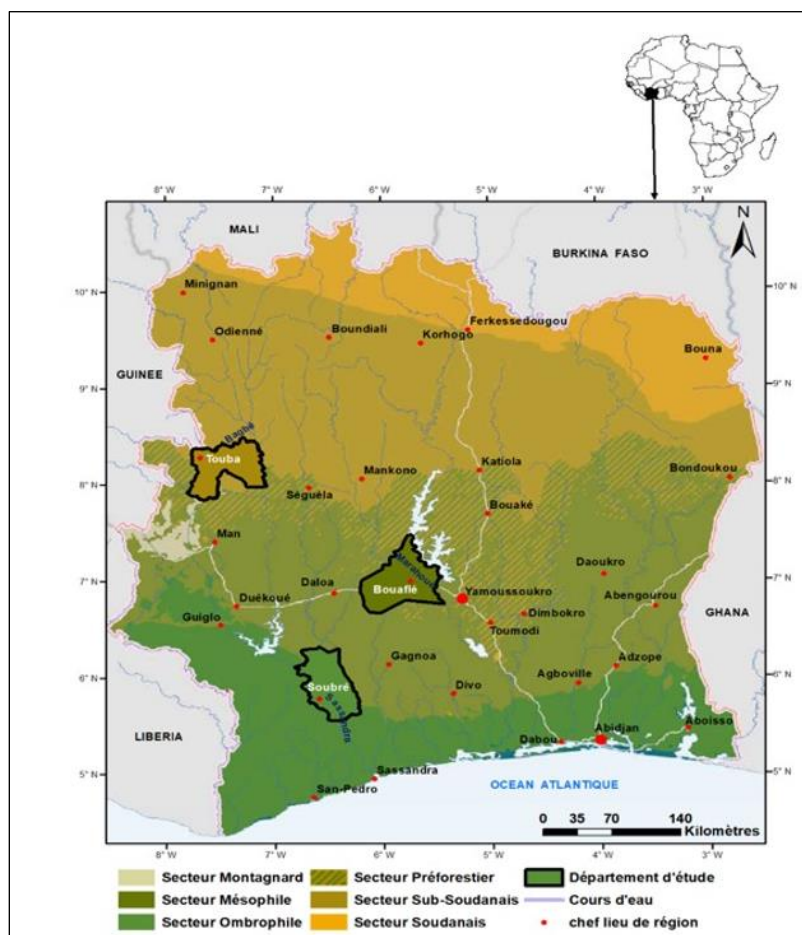


Figure 1: Study areas

2.4 Extraction of genomic DNA from blackflies

Genomic DNA from simuliids was extracted from the whole bodies of the captured individuals using the PrepGEM™ insect DNA extraction kit, in accordance with the manufacturer's instructions. DNA was precipitated by adding 200 μ L of absolute ethanol, and the pellet was dried at room temperature overnight and then resuspended in 75 μ L of elution buffer. The DNA extracts were then stored at -20 °C until they were used for molecular analysis.

2.5 Amplification of PCR products

The genetic structure of *Simulium damnosum* s.l. populations was analysed using microsatellite markers developed specifically for this species complex by ¹⁰. Four microsatellite loci were used; the primer characteristics are presented in Table I. The polymerase chain reaction (PCR) was carried out in a final volume of 25 μ L, comprising 16.3 μ L of molecular water, 2.5 μ L of $MgCl_2$ and 10X buffer mixture, 1.5 μ L of 200 mM dNTPs, 1 μ L of each primer and 0.2 μ L of Taq polymerase (1 U). To this mixture, 2.5 μ L of extracted genomic DNA was added. For each amplification run, a negative control was included by replacing the DNA with 2.5 μ L of sterile water. The reactions were carried out in a thermal cycler according to the following programme: an initial denaturation at 92 °C for 5 minutes, followed by 40 cycles comprising a denaturation step at 92 °C for 30 seconds and a hybridisation step at 50 °C for 30 seconds. In addition to denaturation and hybridisation, two elongation steps were carried out: an initial elongation at 72 °C for 1 minute and a final elongation at 72 °C for 10 minutes. The amplified DNA fragments were separated by size on a 2 per cent agarose gel placed in an electrophoresis tank containing 0.5X TBE running buffer. For each sample, 5 μ L of amplicon was mixed with 3 μ L of 1X loading dye, then dispensed into the gel wells. Electrophoresis was carried out for 45 minutes at 100 V, in the presence of a molecular weight marker of 100 pb. The DNA bands were then visualised using a BioDoc-It System transilluminator.

Table 1: Characteristics of microsatellite markers of *S. damnosum* s.l.

Locus	Repeated sequence	Primers	Designation	Size
60.1	(GT)AT(GT)AT(GT)10	5'-CCCATTTCGCCAGTTGAGGTGA-3' 5'-CCCGTCAACATTGTGGCTACG-3'	SS1 SS2	150 - 975
64.2	(GT)GC(GT)10	5'-ATCATGACGAGGACGCACTC-3' 5'-TACGCACACATTTTCTATTTC-3'	SS3 SS4	110 - 510
7.4	(GT)11TT(GT)	5'-CGCTAACGCTGTGCAATATTG-3' 5'-TGACGAACCTTTGGGACGACA-3'	SS7 SS8	180 - 270
H3-4	(CAG)2(CAA)10(CG)	5'-CGAAAACAACATACGAAGGG-3' 5'-AAATTTTAAATACGTACGAGGG-3'	SS9 SS10p	100 - 500

2.6 Statistical analyses

The genotypic data were entered and organised using Microsoft Excel before being analysed. The databases thus compiled were used to assess the genetic differentiation and structure of the populations studied. A Factor Analytic Correspondence analysis was carried out using the DARwin software, version 6 ¹¹, in order to explore the genetic relationships between individuals and populations. Genetic groupings were then assessed using the Neighbour-Joining method ¹², based on a matrix of genetic dissimilarities. Finally, the optimal number of genetic clusters (K) was determined using the approach described by ¹³, which is based on the evaluation of the logarithmic likelihood of the data, Ln P(D), for different values of K. This approach has made it possible to identify the most likely genetic structure of the *S. damnosum* s.l. populations studied.

3 RESULTS

3.1 Allelic polymorphism of the study populations

Analysis of the electrophoretic profiles of the PCR amplification products (Figure 2) revealed significant allelic polymorphism across all the *S. damnosum* s.l. populations studied. In total, 33 alleles were identified across all the loci analysed, with sizes ranging from 100 to 975 bp. The 100 bp allele was observed in the Touba population, whilst the 975 bp allele was detected in individuals from Bouaflé. Several alleles, notably those of 150, 180, 190, 194, 200, 210 and 220 bp, were widely distributed and were detected at least three loci and in at least two populations. Of these, the 190 bp allele had the highest frequencies at loci 60.1, 64.2 and 7.4, with values of 0.38, 0.55 and 0.45 respectively. The 290 bp allele was particularly common at the H3-4 locus, with a frequency of 0.58. Conversely, certain alleles, notably those of 100, 170, 178, 270, 290, 500, 510, 560, 603, 872 and 975 bp, showed a restricted distribution. These alleles were detected in only one population and were represented by one or two individuals.

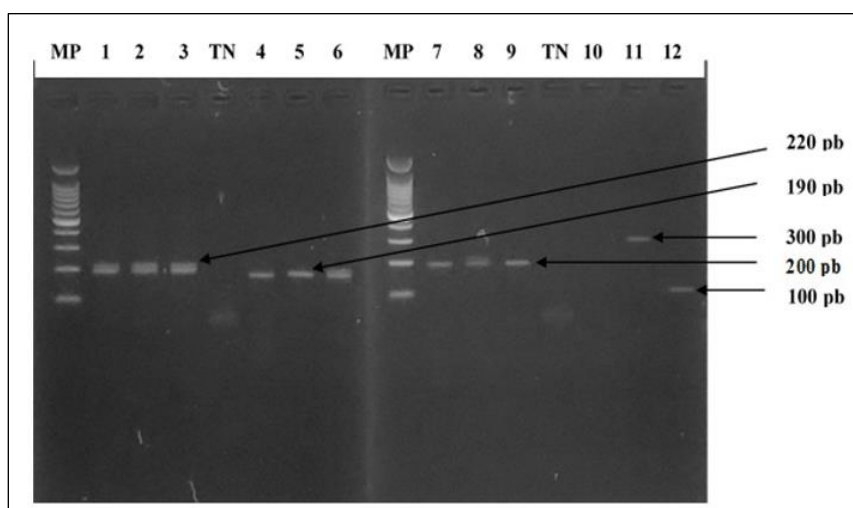


Figure 2: Allelic polymorphism at microsatellite loci 60.1 and 64.2 in *S. damnosum* s.l., as revealed by electrophoresis.

3.2 Genetic structure revealed by factor correspondence analysis

AFC analysis showed that the first two principal axes of the factorial plan account for 50.79 per cent of the total genetic variance (Figure 3). The first axis accounted for 34.68 % of the total variance, and the second for 16.11 %. The projection of individuals onto this factorial plane revealed a genetic structure consisting of two distinct groups (GI and GII), each comprising individuals from the three geographical zones studied. Each group included blackflies collected in both forest and savannah facies. However, both groups consisted mainly of blackflies from savannah areas, with only a few individuals from forest areas.

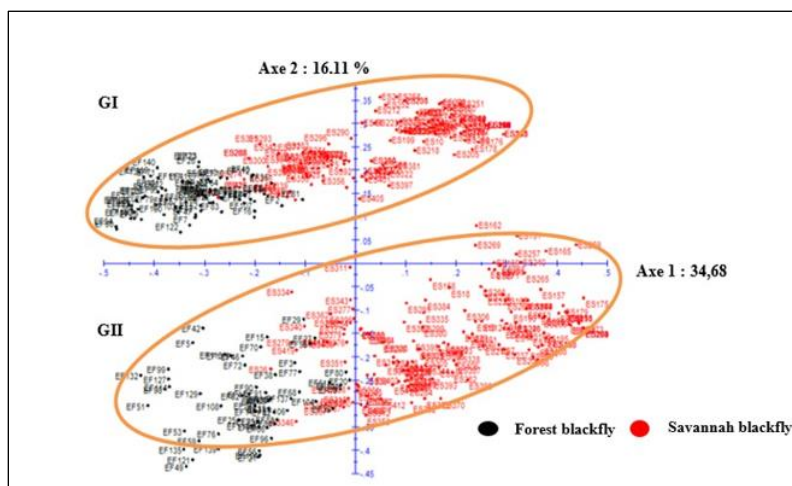


Figure 3: Genetic structuring of *S. damnosum* s.l. populations revealed by the first two axes of a factorial correspondence analysis.

3.3 Genetic classification of individuals

The classification tree obtained from 1,000 bootstrap permutations, with an overall bootstrap value of 0.934 (95 per cent CI: [0.871 – 0.984]), enabled the entire set of genotyped individuals to be divided into four distinct genetic groups (Figure 4). These groups were heterogeneous, each comprising blackflies from savannah and forest, with no clear distinction according to ecological type. The radial representation of the dendrogram displayed a star-like configuration, characterised by relatively weakly differentiated branches among the groups. This organisation suggests a low overall of genetic structure within the *S. damnosum* s.l. populations studied, with limited differentiation between individuals from different ecological facies.

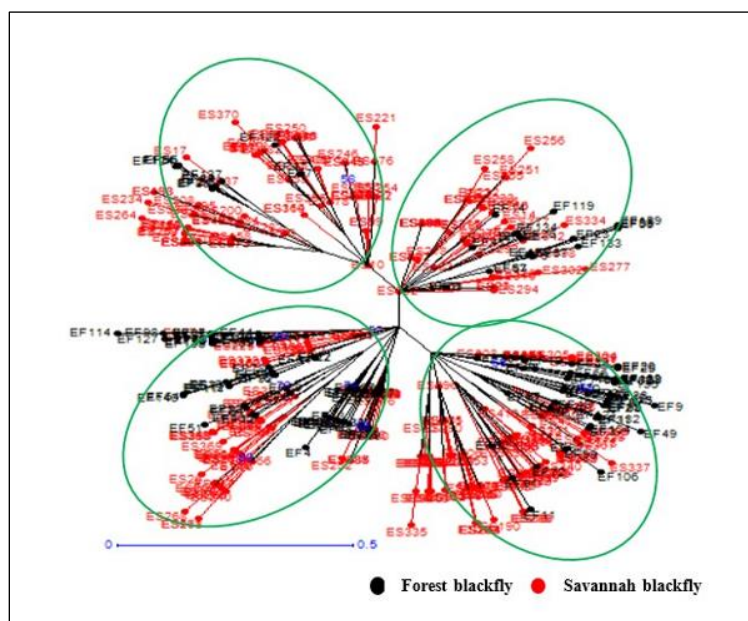


Figure 4: Neighbour-joining tree showing the genetic structure of *S. damnosum* s.l.

3.4 Analysis of genetic structuring by the Evanno method

The Evanno method indicated an optimal number of genetic clusters of $K = 3$, corresponding to the maximum value of ΔK (approximately 8). The assignment of each genotyped individual to the three clusters is shown as a bar plot (Figure 5), in which each individual is represented by a vertical bar whose colour proportions correspond to its membership coefficients in the different clusters. Of the 437 individuals genotyped, 196 (44.85%) were assigned mainly to cluster A (red), 136 (31.12%) to cluster C (green) and 105 (24.03%) to cluster B (blue).

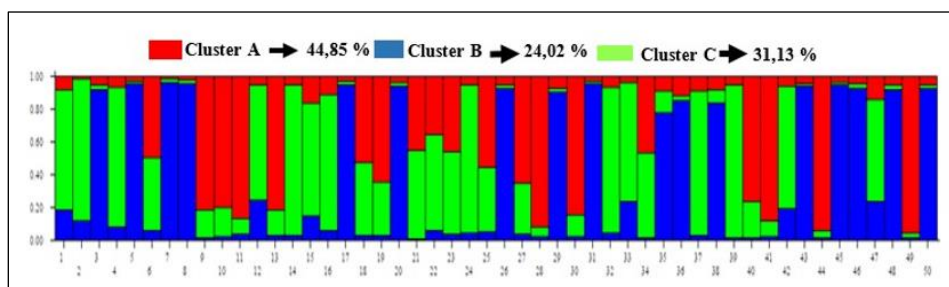


Figure 5: Genetic assignment of *Simulium damnosum* s.l. individuals to three clusters ($K = 3$) inferred with STRUCTURE

3.5 Genetic differentiation of the populations of *Simulium damnosum* s.l

The analysis of the genetic differentiation of the populations of *Simulium damnosum* s.l. based on estimation of the Wright Fixation index (F_{ST}) from 5,000 bootstrap permutations on all the analyzed loci, revealed a global F_{ST} value of 0.12 (CI 95 % : [0,053 – 0,132]) (Table 2). This value indicates a moderate genetic differentiation between the studied groups. Group C has the highest F_{ST} value ($F_{ST} = 0.230$), reflecting a relatively important genetic differentiation compared to the other groups. Conversely, Group B was characterized by a very low F_{ST} value ($F_{ST} = 0.006$), reflecting a low genetic differentiation between the individuals who compose it.

Table 2: Genetic diversity between the three groups according to the Weir and Cockrham method

	Effectifs	Ho	He	F_{ST}
Group A	196	0.406	0.656	0.161
Group B	105	0.329	0.755	0.066
Group C	136	0.264	0.578	0.230
Mean	437	0.333	0.663	0.152

4 DISCUSSION

The results reveal high genetic diversity within *Simulium damnosum* s.l. populations, with 33 alleles identified across all loci analysed. Widely distributed alleles, notably the 190 bp allele, co-occurred with rare alleles restricted to particular populations, which suggests both the sharing of genetic variants among populations and some degree of local differentiation. The high frequency of the 190 bp allele at several loci, and of the 290 bp allele at locus H3-4, points to substantial sharing of genetic diversity among the populations studied. This is consistent with recent RADseq results for *S. damnosum* s.l. in northwestern Ethiopia, where differentiation between some populations coexisted with individuals whose genetic profiles were associated with other localities, suggesting connectivity⁴. However, multivariate and clustering analyses revealed a genetic structure that does not clearly correspond to the forest-savannah ecological gradient. Correspondence analysis (CA) separated two genetic groups, whereas the Bayesian clustering analysis identified three clusters ($K = 3$), and each of these groups included individuals from both forest and savannah areas and from the different geographical zones sampled. The absence of grouping by habitat suggests that the forest-savannah gradient is not a major barrier to gene flow among the populations studied. This agrees with the overlap in the distribution of certain cytoforms of *S. damnosum* s.l. between forest and savannah environments reported in Cameroon³. The distribution of individuals among the three clusters points the same way: 44.85 % of individuals were assigned mainly to cluster A, 31.12 % to cluster C and 24.03 % to cluster B. Such an organization suggests that the populations studied are not strictly isolated genetic units and may reflect gene flow that limits differentiation despite geographical distance or ecological differences. A comparable pattern was reported by⁴, where individuals from different localities were assigned to the same genetic cluster, which the authors interpreted as an indication of migration

and connectivity. The dendrogram provides complementary evidence: its groups were heterogeneous and showed no clear separation between forest and savannah areas. The star configuration and the relatively little differentiated ramifications indicate a limited global structuring, despite the existence of genetic differences between certain individuals or groups. This organization is compatible with the results of ⁴, which also highlighted differentiation between certain genetic components, but a weak differentiation within clusters and a grouping of individuals from different localities. The observed structure could result from several demographic and evolutionary processes. The coexistence of different genetic groups within the same ecological zones may in particular be compatible with a history of mixing between populations. A Wahlund effect could also contribute to the structuring observed if several genetically differentiated subpopulations reproduce locally within the same sampled ensembles. However, this hypothesis cannot be accepted solely on the basis of the dendrogram and should be confronted with the results of the genotypic frequencies and the Hardy-Weinberg equilibrium. Likewise, the possible coexistence of several ecotypes within the *S. damnosum* s.l. could contribute to the observed structure, but this interpretation requires additional data to distinguish a real ecological differentiation from a simple demographic structuring ¹⁴. However, the global F_{ST} brings an important nuance to this apparently poorly structured organization. The value of $F_{ST} = 0.12$, indicates a moderate genetic differentiation between the studied groups. Thus, despite the sharing of many alleles, the strong admixture and the absence of a clear separation according to the ecological facies, the populations retain a measurable genetic differentiation. This observation suggests that the connectivity between populations could be sufficient to favor the sharing of certain variants without however leading to a complete homogenization of their allelic frequencies. The variation in the values of F_{ST} between the groups reinforces this interpretation. Group B has a very low differentiation ($F_{ST} = 0.006$), while group C has the highest value ($F_{ST} = 0.230$). This heterogeneity indicates that genetic differentiation is not uniform within the overall structure and could reflect different demographic histories or levels of connectivity between the genetic components. However, these values must be interpreted with caution, since their meaning depends in particular on the definition of the groups, their size and the loci considered. Overall, the results indicate that the populations of *S. damnosum* s.l. studied exhibit a high genetic diversity, associated with a partial genetic structuring and a strong admixture. The absence of a clear separation between the forest and savanna facies, combined with the grouping of individuals from different geographical areas within the same genetic components, suggests an important connectivity between populations. At the same time, the moderate differentiation revealed by the F_{ST} shows that this connectivity does not lead to a complete homogenization of the populations. This organization could result from the combined action of vector dispersion, gene flow and local demographic processes. From an epidemiological point of view, this potential connectivity is important, since it could favor the circulation of vector populations between different transmission zones and thus contribute to maintaining the genetic continuity.

5 CONCLUSION

This study reveals a high genetic diversity and a partial genetic structuring of the populations of *Simulium damnosum* s.l., without a clear separation between the forest and savanna facies. The high admixture and the grouping of individuals from different geographical areas suggest an important genetic connectivity between populations, while the global F_{ST} indicates a moderate genetic differentiation. These results underline the importance of integrating the genetic characterization of *S. damnosum* s.l. populations in the surveillance of onchocerciasis vectors in Côte d'Ivoire. They also provide a basis for deepening the study of connectivity between transmission foci and mechanisms likely to promote the circulation of vector populations. Analyses based on a large number of genomic markers and a wider spatial and temporal sampling would make it possible to specify the origin of the observed structuring and to better characterize gene flows between populations.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Authors' contribution

SS conceptualisation, investigation, formal analysis, writing original draft, **SS** formal analysis and writing-review, **AAB**, **TM**, **GBK** and **AIA** review and editing, **AAB** formal analysis, supervision, review and editing. The authors declare that they have no conflicts of interest.

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