Genetic Diversity of *Simulium damnosum* complex Onchocerciasis Vector and its Influence on Entomological Monitoring in the West of Côte d'Ivoire

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Abstract

In West Africa, *Onchocerca volvulus*, the causative pathogen of human onchocerciasis, is transmitted by sibling species of the *Simulium damnosum* complex. Little is known about blackfly intraspecific variability and its consequences on vectorial capacity in Côte d'Ivoire. This study reports the use of microsatellite markers to study the genetic profile and evaluate the gene flow between populations of *S. damnosum s.l* from three (3) different epidemiological facies in western Côte d'Ivoire, fifteen years after the end of onchocerciasis control program. Adult flies were collected on human attractants from 07 : 00 to 18 : 00 hours for three consecutive days by site, from December 2016 to October 2017. Four (4) microsatellite loci were used to characterize individuals from these populations. The four (4) loci were polymorphic with 13.25 alleles per locus. Two (2) specific alleles (190 bp and 290 bp), were reveal abundant with respective frequencies of 0.46 % and 0.58

% . A significant heterozygosity deficiency and low genetic differentiation (F_{ST} = 0.046, P = 0.024) have been observed for all the populations. The genetic analysis showed significant deviation from Hardy-Weinberg and significant heterozygosity deficiencies. Then, the great interspecific variability would be a general characteristic in *S. damnosum s.l.* At last, a probable evolution of the invasive strains of *S. damnosum* would have occurred in these localities. This study has shown significant deviation from Hardy-Weinberg and significant heterozygosity deficiencies in the populations of the three localities. The loci give independent estimate genetic parameters. The H3-4 locus to a low genetic differentiation between the populations.

Keywords: Onchocerciasis, *Simulium Damnosum S.L*, SSR Markers, Polymorphism, Genetic Differentiation

Introduction

Human onchocerciasis, or river blindness, results from infection with the filarial parasite *Onchocerca volvulus*. In West Africa, the incidence of blindness due to infection with *O. volvulus* is much higher in savannah than in forest areas (Prost, 1980). The rate of blindness can reach up to 15 % in savannah, whereas in forest zones, ocular manifestations of the disease are less severe, with a much lower degree of blindness (Duke, 1990). In West Africa, blackflies (Diptera: Simuliidae) of the *Simulum damnosum* complex serve as vectors for *O. volvulus* (Prost, 1980). From 1968, the international community mobilized human financial resources, material and technical to fight against this disease (Hougard *et al.,* 2001). Then, from 1974 to 1990, the World Health Organization set up a vast onchocerciasis control program called OCP (WHO, 2002), which partly covered the endemic countries of West Africa. This strategy has reduced the transmission of the disease below the threshold for a public health problem in the majority of treated areas of covered countries, including Côte d'Ivoire (WHO / APOC ; Sightsavers, 2011). However, after stopping of OCP activities in 2002, Côte d'Ivoire was unable to conduct regular control activities because of the socio-political crisis which had just begin in September 2002. The interruption of larviciding treatments in vector control has certainly exposed resident populations to more and more abundant flies bites (WHO, 2002). OCP objective did not aim the eradication of the parasite and its vector and risk factors for recrudescence of the disease do still exist (Adjami *et al.,* 2006). Therefore, the knowledge of the biological characteristics of *S. damnosum s.l* populations in view of climatic and environmental changes, but also the determination of the differentiation scale and the estimation of gene flow between populations is necessary. A wide range of molecular markers has been used for population studies. These include chromosomal inversions, allozymes, random amplified DNA

polymorphisms, mitochondrial DNA sequences, and microsatellite loci analysis (Simard *et al.,* 1999). Microsatellites are informative polymorphic DNA markers that are widely used to examine genetic diversity in the population of *anopheles funestus* (Cohuet *et al.*, 2005), *Aedes aegypti* (Ravel *et al.,* 2001 ; Ravel *et al.,* 2002), *Triatoma dimidiate* (Anderson *et al.,* 2001), *Glossina palpalis* (Solano *et al.,* 1999), and *Simulium damnosum s.l* (Dumas *et al.,* 1998). In this study we focus on microsatellite to dicipher the genetic diversity of *S. damnosum s.l* and evaluate the gene flow between populations of *S. damnosum s.l* in the context of climate and environmental changes.

Material and Methods

Capture biting adult females of *S. damnosums s.l.*

The classical capture method at human's bait (Le Berre, 1966 ; Philippon, 1977 ; Simaro *et al.,* 2019) was used for collection of blackflies. A team of two people accomplished the capture of blackflies, one hour per person. The daily collected specimens was separated into clockwise period, and the flies capture tube containing one fly per tube, were labeled with the location, date and time of capture. The catches were made from 7 am to 6 pm for 3 days by site. One site per locality was investigated and the individuals capturing were located 20 m from the river. Specimens were collected from December 2016 to October 2017.

Morphological identification of female blackflies

Morphological parameters such as antenna and bristles of the wing tuft colors ; the colors of the arculus ; the scutellum bristles ; the first article of the anterior paw or procoxa and the bristles of the 9th abdominal tergite were examined (Quilievéré., 1976 ; Simaro *et al.,* 2019). The combined analysis of these parameters made it possible to identify savana female blackflies (*S. damnosum ss* and *S. sirbanum*), and forest females (*S. yahense, S. sanctipauli, S. squamosum* and *S. soubrenses*).

DNA extraction from blackflies specimans

Simulium damnusum s.l DNA was extracted from the whole body of adult female, according to a protocol based on NaCl buffer and adapted by the Research Unit of Genetics and Molecular Epidemiology (URGEM) of Jean Lorougnon Guede University. Briefly, the blackflies were manually crushed in a 1.5 ml eppendorf tube containing 200 µl of red blood cell lysis buffer. The mixture was then incubated at 56 °C for 1 h to lyse the proteins. A centrifugation at 12 000 rpm for 30 min permit to recover the solubilized DNA in the aqueous phase. The DNA is then precipitated in 200 µl of absolute ethanol and then deposited by centrifugation for 20 minutes at 12 000 rpm.

The DNA deposit is then dried at room temperature overnight and re-suspended in 75 µl of TE buffer.

DNA amplification with microsatellite markers

Primers developed previously for *S. damnosum s.l* (Dumas *et al.,* 1998) were used for Polymerase chain reaction (PCR) (Table 1). PCR was carried in 50 µl final volumes, containing 2.5 µl of $MgCl_2$ with 10X reaction buffer, 1.5µl of desoxyribonucleotide (dNTP 200 µM), 1 µl of each primer, 1 units of Taq DNA polymerase, 2.5 µl of ADN sample and 16.3 µl molécular water. After an initial denaturation at 92 °C for 5 min, samples were processed through 40 cycles consisting of a denaturation step at 92 °C for 30 s, an annealing step at 50 °C for 30 s, and an extension step at 72 °C for 1 min. The final elongation step was lengthened to 10 min. Amplification products were checked by electrophoresis on 2 % agarose gels immersed in 0.5X TBE buffer. 5 µl of each amplified sample is mixed with 2 µl of loading buffer (glycerol 50 %, bromophenol blue 0.2 %, xylene cyanol 0.2 %, EDTA pH 8 0.2M). A ΦX174 size marker was used to quantify allele size. After 45 minutes of electrophoretic migration, the gel is visualised with a gel viewer (BioDOC-IT System) under UV light.

Table 1: Microsatellite loci, forward and reverse primer sequences.

Locus	Repeat sequence	Primers Sequence (5' → 3')	Designation	Product length (bp)
60.1	$(GT)AT(GT)AT(GT)_{10}$	CCCATTTGCCAGTTGAGGTGA CCCGTCAACATTGTGGCTACG	SS1 SS2	975
64.2	$(GT)GC(GT)_{10}$	ATCATGACGAGGACGCACTC TACGCACACATTTTTCTATTTC	SS3 SS4	510
7.4	$(GT)_{11}TT(GT)$	CGCTAACGCTGTGCAATATTG TGACGAACTTTGGGACGACA	SS7 SS8	270
H3-4	$(CAG)_2(CAA)_{10}(CAG)$	CGACAACGTGTCTCGACAAA CGAAAACAACATACGAAGGG	SS9 SS10p	500

Data analysis

Test for linkage disequilibrium and Genetic variability parameters for each population (number of distinct alleles, observed and expected heterozygosities under Hardy-Weinberg equilibrium), were conducted using GENETIX software, version 4.05.1 (Belkhir *et al.,* 2004). The Fstat software version 2.6.4 (Goudet, 2003), is used to estimate the heterozygosity deficiencies of individuals in their sub-population (F_{IS}), and simulated distribution of heterozygosity between a pair of populations (F_{ST}). The FDIST2 software (Beaumont et Nichols, 1996) was used to identify markers submitted for natural selection.

Results

Simulian fauna

A total of 4244 blackflies were captured during the survey. Two groups of Simulium species have been identified. The savanna group species (*Simulium damnosum ss*, and *S. sirbanum*) abundant in Touba and Bouaflé with 2900 blackflies captured, and the forest group species (*S. squamosum*, *S. sanctipauli*, *S. soubrense*) present only in Soubré with 1103 blackflies.

Genetic polymorphism of *s. damnosum s.l* populations

Electrophoretic profiles (Fig.1) obtained after the PCR reveals 33 different alleles in the whole populations. 17 alleles were observed at the 60.1 locus, 12 alleles at the 64.2 locus, 11 alleles at the 7.4 locus and 13 alleles at the H3-4 locus, with an average number of 13.25 alleles per locus. The alleles size vary from 100 bp at the H3-4 locus to 975 bp at the 60.1 locus. A specific allele 190 bp is reveled at the loci 60.1, 64.2 and 7.4, with respective frequencies of 0.38 %, 0.55 %, 0.45 %. Similarly, the 290 bp allele is reveled abundant to the H3-4 locus with a frequency of 0.58 %

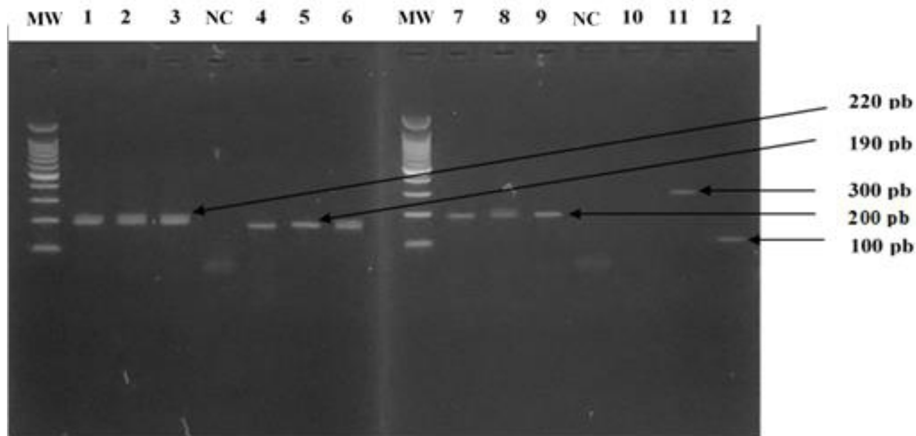

Fig 1: Electrophoregram on 2 % agarose gel.
Abbreviation: MW: Molecular weight marker (72 bp) ; 1, 2, 3, 6, 8: Sample of heterozygous profile ; 4, 5, 7, 9, 11, 12: Homozygous profit sample ; NC: Negative control

Linkage disequilibrium within the three zones

The exact test of the linkage disequilibrium between all the loci in each sample made it possible to analyze a total of 961 allelic combinations. No significant values were obtained across all populations for any locus combination ($P > 0.05$ for each pairwise comparison), suggesting that the loci give independent estimate of population genetic parameters.

Genetic variability among populations

Mean observed heterozygosity per locus, varied between 0.346 and 0.428 respectively in Soubré and Bouaflé. Which is lower than expected heterozygotes (0.710 for Soubré and 0.576 for Touba) (Table 2). The four polymorphic loci, showed significant deviation from Hardy-Weinberg. Moreover, the F_{IS} values were positive at all loci, indicating extensive heterozygosity deficiencies.

Table 2: Genetic diversity and intrapopulation differentiation

Locus		Touba	Bouaflé	Soubré	*P*-value
	N	147	151	139	
	n	8	10	12	
60.1	**H_o**	0.429	**0.543**	0.469	0.0139
	H_e	0.631	0.724	0.817	0.0239
	F_{IS}	0.321	0.251	0.426	0.0466
	N	6	7	9	
64.2	**Ho**	0.282	0.377	0.260	0.0252
	H_e	0.480	0.680	0.721	0.0525
	F_{IS}	0.415	0.446	0.639	0.0619
	N	8	7	7	
7.4	**Ho**	0.333	0.385	0.379	0.0272
	H_e	0.586	0.684	0.771	0.0567
	F_{IS}	0.433	0.438	0.510	0.0625
	N	7	9	6	
H3-4	**Ho**	0.371	0.408	**0.276**	0.0264
	H_e	0.609	0.593	0.532	0.057
	F_{IS}	0.392	0.312	0.482	0.061
Moyenne	**N**	29	33	34	
	Ho	0.353	**0.428**	**0.346**	0.011
	H_e	0.576	0.670	**0.710**	0.0145
	F_{IS}	0.3879	0.3616	0.5134	**0.0314**

Abbreviation: N **:** number of individuals tested in the population ; n : number of alleles ; H_o **:** heterozygote observed ; H_e : Heterozygote expected ; *P*-value : probability of conformity to the Hardy-Weinberg equilibrium

Genetic differentiation of *S. damnosum s.l* populations

The exact test for population differentiation indicated that a low genetic differentiation is observed, when comparing F_{ST} between the three populations (Table 3), suggesting that genotypes of the individuals result from the same gene pool. The overalls F_{ST} were 0.025, 0.007 and 0.016 for pairwise populations of Touba and Bouaflé, Touba and Soubré, and Soubré and Bouaflé, respectively. In the same way, populations of Touba and that of Bouaflé are genetically close to from each other (F_{ST} = 0.025).

Table 3: Genetic Differentiation in *S. damnosum* Populations

Loci	60.1	64.2	7.4	H3-4	all loci	*p*-value
Touba/ Bouaflé	0.032	0.022	0.010	0.037	0.025	0.057
Touba / Soubré	0.090	0.042	0.038	0.113	0.071	**0.007**
Soubré / Bouaflé	0.049	0.030	0.038	0.052	0.042	**0.016**
Total	0.057	0.031	0.028	0.067	**0.046**	**0.024**

Markers under selection

Analysis of F_{ST} between populations of *S. damnosum s.l*, indicates that no microsatellite loci are submitted to the natural selection (Fig. 2). When we are around the median (expected F_{ST} under the neutral hypothesis), two (2) markers 64.2 and 7.4 showed low levels of genetic differentiation between populations. In contrast the 60.1 and H3-4 loci showed a moderate level of genetic differentiation, but the H3-4 locus would contribute more to this genetic differentiation between populations.

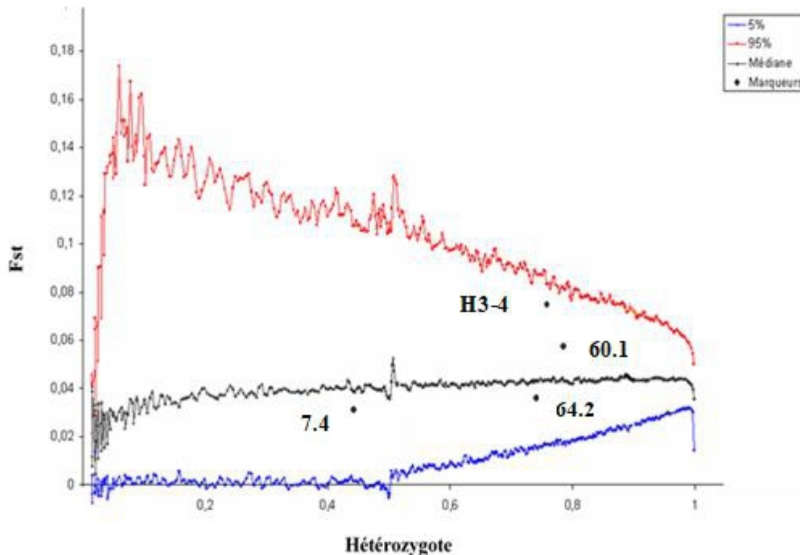

Fig. 2: Expected Fst values for heterozygosity on four (4) microsatellite markers at three (3) populations of *Simulium damnosum s.l*

Discussion

All of the blackflies captured in this study belong to the *S. damnausum sl* complex as reported by previous studies in other localities of the country, especially at the ecological station of Taï and the site of the dam of Soubré (Traore *et al.,* 1980 ; Traore and Hebrard, 1981 ; Yapi *et al.,* 2014). Specific identification indicates that savanna species constitute essential of the catches in the sites of Bouafle and Touba. With regard to the site of Soubre, forest species are the only ones encountered. Indeed, Bouafle borders the "V

Baoule", advanced profound towards the South, in the forest. In relationship with deforestation and declining rainfall in recent decades, we are witnessing a gradual reduction of extent of the wooded areas and southern wet. This induce the progression of the species distribution area of *S. damnosum* vectors of savanna onchocerciasis towards the south where they substitute, sometimes massively, and more and more durably, to native species wetlands. However, the group of savanna blackflies (*S. damnosums, S. sirbanum*) and savannah *O. volvulus* form one of the most effective couples in onchocerciasis transmission (WHO, 2002).

The microsatellite loci used to evaluate the level of genetic variation, were highly polymorphic in the three populations, ranging from 11-17 alleles per locus. This number is accordance with results obtained in two *S. damnosum s.l* populations study of Mali (Dumas *et al.,* 1998), in which 8-12 mean alleles per locus were found. Also, the few studies of *S damnosum s.l* at population level using molecular markers (Adjami *et al.,* 2006) indicate, in general, a great interspecific variability. This high level of genetic polymorphism would be a general characteristic in *S. damnosum s.l.* No linkage disequilibrium between loci was detected in any population, suggesting that the loci give independent estimates of population genetic parameters. Similar studies of other tropical disease vectors corroborate this result. It's about especially works of Acapovi-Yao *et al.* (2015) on the genetic differentiation and structuration of *Glossina palpalis palpalis* populations in Azaguié zone (Côte d'Ivoire), and Akré *et al.* (2015), on the genetic structure of *Anopheles nili s.s* vector populations of malaria in rural and peri-urban of Côte d'Ivoire.

Genetic analysis of *S. damnosum s.l* populations showed significant deviation from Hardy-Weinberg and significant heterozygosity deficiencies. Such deficiencies would be generated by numerous factor, including inbreeding, population structure and null alleles. Indeed, the analysis of 7.4 and H3-4 markers reveals the failure of PCR amplification was due to point mutation in the primer. So, the fact that some alleles have not amplified at some loci, makes the presence of null alleles is the most explanation for these deficits of heterozygosity. These results are in agreement with the study of Dumas *et al.* (1998). They also demonstrated the existence of null alleles during their study on the use of microsatellite markers to differentiate two savanna populations in Mali. Also, inbreeding could also be the cause of this deficiency, but it seems unlikely, since such effects should be expected to be evident for all loci (Dumas *et al.,* 1998). Structuration of the population is another cause of the heterozygosity deficiencies observed. Of the three studied sites, two are within the area of OCP control, where blackflies populations have been regularly eliminated through insecticides treatments. If recolonization occurs and is very recent, a Walhund effect could ensue

(Adjami *et al.,* 2006). This would produce heterozygosity deficiencies, if the colonizing populations are markedly differentiated (Dumas *et al.,* 1998). Another explanation could be the presence of mixed species in these populations. (Estrada-Franco *et al.,* 1992) demonstrated that an excess of homozygotes in *anopheles* populations was due to mixed species of the *Anopheles quadrimaculatus* complex, leading to the description of a new species.

The study performed by comparing two by two samples of *S. damnosum s.l*, has shown the existence of a low genetic differentiation between these populations. This result could be explained by high migration rates, estimated from null alleles (Slatkin, 1985). The results obtained in this study confirms the preceding study of Adjami *et al.* (2006), which tend to show that the migration is an essential characteristic of *S damnosum s.l.* Also, the high dispersal ability of *S. damnosum s.l* in combination with high migration rates, permitted a genetic mixing which tends to homogenize populations and limit their differentiation. However, mean pairwise F_{ST} low significance between Western Simulium populations from Côte d'Ivoire, would be due to a probable evolution of the main of invasive *S. damnosum* strains in these localities. This evolution would have occurred during the migrations, which would have favored genetic exchanges between Simulium populations.

The microsatellite markers used in this study do not show natural selection signatures in *S. damnosum s.l.* populations. However, the H3-4 locus with a moderate differentiation index would be the region of the genome susceptible to be involved in the long term, in a probable adaptation of Simulium to very diverse environmental conditions across its distribution area. This is the approach of (Beaumont and Nichols, 1996), which is based on the idea that in a population, all genome loci have same F_{ST}. Only the loci under selection derogate from this rule, because the natural selection edit their F_{ST}.

Conclusion

This study showed significant deviation from Hardy-Weinberg and significant heterozygosity deficiencies in the populations of the three localities. Then the hypothesis of a Wahlund effect is also suggested. Also, the study of linkage disequilibrium between loci indicates that, the loci give independent estimate genetic parameters. The H3-4 locus is not systematically involved in the genetic differentiation observed. Since then, only constraints specific to this locus would be at the origin of the differences observed. The presence of null alleles is revealed at this locus. Finally, this locus contributes by the value of its F_{ST} to a low genetic differentiation between the populations. Complementary studies will be carried out to deepen knowledge on the genetic structure of populations of this vector onchocerciasis in Côte d'Ivoire.

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