Population Genetics as a Tool to Select Tsetse Control Strategies: Suppression or Eradication of *Glossina palpalis gambiensis* in the Niayes of Senegal

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**Abstract**

*Background:* The Government of Senegal has initiated the “Projet de lutte contre les glossines dans les Niayes” to remove the trypanosomosis problem from this area in a sustainable way. Due to past failures to sustainably eradicate *Glossina palpalis gambiensis* from the Niayes area, controversies remain as to the best strategy implement, i.e. “eradication” versus “suppression.” To inform this debate, we used population genetics to measure genetic differentiation between *G. palpalis gambiensis* from the Niayes and those from the southern tsetse belt (Missira).

*Methodology/Principal Findings:* Three different markers (microsatellite DNA, mitochondrial CO1 DNA, and geometric morphometrics of the wings) were used on 153 individuals and revealed that the *G. p. gambiensis* populations of the Niayes were genetically isolated from the nearest proximate known population of Missira. The genetic differentiation measured between these two areas (ΦST = 0.12 using microsatellites) was equivalent to a between-taxon differentiation. We also demonstrated that within the Niayes, the population from Dakar – Hann was isolated from the others and had probably experienced a bottleneck.

*Conclusion/Significance:* The information presented in this paper leads to the recommendation that an eradication strategy for the Niayes populations is advisable. This kind of study may be repeated in other habitats and for other tsetse species to (i) help decision on appropriate tsetse control strategies and (ii) find other possible discontinuities in tsetse distribution.

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**Introduction**

The Niayes of Senegal harbours the most northern and western population of *Glossina palpalis gambiensis* Vanderplank, which is a major vector of the debilitating diseases Human African Trypanosomosis (HAT) or sleeping sickness, and African Animal Trypanosomosis (AAT) or nagana (reviewed in [1]). Particular meteorological and ecological characteristics of this area provide great potential for agricultural development in general and animal production (cattle, donkeys, horses, small ruminants, pigs and poultry) in particular. Most of these animals are however susceptible to AAT which is seriously limiting the development of efficient and productive, sustainable livestock systems. The socio-economic impact of the disease is therefore dramatic and very often underestimated [2]. In the 1970s and 1980s, it was attempted to eliminate the *G. p. gambiensis* population from the Niayes mainly using ground spraying of residual insecticides [3]. The tsetse and trypanosomosis problem seemed to have disappeared until flies were detected again in 1998 (unpublished report of the Direction de l’Élevage - DIREL). In 2005, the DIREL initiated a control campaign called “Projet de lutte contre les glossines dans les Niayes” with the objective of developing a sustainable solution to the tsetse and trypanosomosis problem in the Niayes. The programme is funded by the Government of
Problem.

Increased efforts to manage the tsetse and trypanosomosis

The level of isolation of the targeted tsetse populations will be an

Several factors. The Government of Senegal wants to

Sustainably eliminate trypanosomosis from the Niayes

Several strategies and methods that can be used for

Control of these vectors is a very efficient way to contain these diseases. There are

Several factors. The Government of Senegal wants to sustainably eliminate trypanosomosis from the Niayes region by controlling the tsetse vector, Glossina palpalis gambiensis. To reach this objective, two different strategies may be used: suppression (decrease in tsetse densities) or eradication (remove all the tsetse in the region until last one). For eradication, the approach has to be area-wide, i.e. the control effort targets an entire pest population within a circumscribed area, to avoid any possible reinvasion. Three different tools (microsatellite DNA, mitochondrial DNA and morphometrics) were used, and all showed an absence of gene flow between G. p. gambiensis from the Niayes and from the nearest known population in the south east of the country (Missira). This genetic isolation of the target population leads to the recommendation that an eradication strategy for the Niayes populations is advisable. This kind of study may be extended to other areas on other tsetse species.

Methods

In the Niayes region, four tsetse populations were sampled using Vavoua traps: Dakar Hann which is a swamp forest harbouring an animal park within the city of Dakar, Diacssaw Peul, an area of riparian thicket where tsetse and cattle are in intense contact, Sebikoton and Pout, which are mango and citrus-tree plantations where tsetse and people are in close contact. The tsetse flies collected in the Niaye area and analyzed in the present study are the sole property of the Senegalese authorities. They were collected by the national veterinary services through official mission orders, in one wildlife park (Parc de Hann) and three private sites, with the oral consent of the owners. No written consent is mandatory for tsetse fly collection in Senegal. In the south-eastern part of the country, the area of Missira was sampled: it is the nearest known infested area from the Niayes, according to a detailed tsetse survey implemented as part of the baseline data collection of the Niayes tsetse Control project (see Fig. 1). Areas between the Niayes and Missira are not favourable for tsetse, which was confirmed by zero tsetse catches (JB, BS unpublished data) despite intensive trapping efforts.

In total 153 tsetse individuals were analysed originating from Diacssaw Peul (22 females (F), 8 males (M)), Dakar Hann (23F, 6M), Sebikoton (21F, 11M), Missira (23F, 12M), and Pout (13F, 14M).

Microsatellite DNA markers

Markers used and PCR conditions. A total of 10 microsatellite markers were used (preceded by “X” for X-linked loci): X55.3 [21], XPgp11, Pgp1, XPgp13, Pgp24 [22], A10, XB104, XB110, C102 (A. Robinson, FAO/IAEA, pers. com.) and GPCAG [23]. The samples were then processed for Polymerase Chain Reaction (PCR) and genotyping on a 4300 DNA Analysis System from LI-COR (Lincoln, NE) exactly as described in [19].

Population structure analyses. Wright’s F-statistics [24], the parameters most widely used to describe population structure [25], were initially defined for a three-levels hierarchical population structure (individuals, sub-populations and total). In such a structure, three fixation indices or F-statistics can be defined. $F_{IS}$ is a measure of the inbreeding of individuals (hence I) resulting from non random union of gametes within each sub-population (hence S). $F_{ST}$ is a measure of the relatedness between individuals resulting from non-random distribution of individuals among sub-populations, relative to the total population; $F_{IT}$ quantifies the differentiation between sub-populations in the total population (hence S and T). $F_{IT}$ is a measure of the inbreeding of individuals resulting both from non-random union of gametes within sub-populations and from population structure (deviation from panmixia of all individuals of the total population, hence I and T). These F-statistics are classically estimated by Weir and Cockerham’s unbiased estimators $f$ for $F_{IS}$, $\theta$ for $F_{ST}$ and $F$ for $F_{IT}$ [26]. When appropriate, these statistics were estimated with

Author Summary

Tsetse flies transmit trypanosomes to humans (sleeping sickness) and animals (nagana). Controlling these vectors is a very efficient way to contain these diseases. There are several strategies and methods that can be used for control, each being more or less efficient depending on several factors. The Government of Senegal wants to sustainably eliminate trypanosomosis from the Niayes region by controlling the tsetse vector, Glossina palpalis gambiensis. To reach this objective, two different strategies may be used: suppression (decrease in tsetse densities) or eradication (remove all the tsetse in the region until last one). For eradication, the approach has to be area-wide, i.e. the control effort targets an entire pest population within a circumscribed area, to avoid any possible reinvasion. Three different tools (microsatellite DNA, mitochondrial DNA and morphometrics) were used, and all showed an absence of gene flow between G. p. gambiensis from the Niayes and from the nearest known population in the south east of the country (Missira). This genetic isolation of the target population leads to the recommendation that an eradication strategy for the Niayes populations is advisable. This kind of study may be extended to other areas on other tsetse species.

Senegal and technically and financially supported by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA). The project is implemented in the context of the African Union - Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), a political initiative of the African heads of state that calls for increased efforts to manage the tsetse and trypanosomosis problem.

Tsetse populations may be reduced using a variety of techniques, including insecticide impregnated traps and targets, live-baits, sequential aerial spraying, and sterile male releases [4-9]. In the past, most control efforts were not implemented according to area-wide principles [10,11], and as a consequence, when the control effort was reduced or stopped, the tsetse populations tended to recover—due to either flies surviving the initial interventions, or migrant flies coming from untreated regions, or both [12]. This has fuelled a debate as to whether in such a structure, three fixation indices or F-statistics can be defined, by FAO [13,14] as the creation of a tsetse free zone, may be more cost effective than “suppression” where tsetse densities are reduced to a level facilitating when the population structure within the target region, whether to select an eradication or suppression strategy will be defined.

The level of isolation of the targeted tsetse populations will be an important parameter to guide the Government of Senegal to select the most optimal control strategy. Here we report population genetic analyses of microsatellite and mtDNA markers combined with morphometrics of G. p. gambiensis populations sampled from the Niayes area and from the nearest population in the south-eastern part of the country (fig. 1) to assess their degree of isolation by measuring gene flow among the different populations. The genetic differentiation of the various G. p. gambiensis populations within the Niayes was also assessed to determine if the different populations of the Niayes can be targeted at the same time (if it is a panmictic unit), or if a sequential control strategy can be contemplated (if substantial genetic differentiation between populations is found), which will also depend on their respective history, including effective population sizes and possible bottlenecks.

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Figure 1. Geographic location of the study sites in Senegal. The study sites are located on the map, together with putative tsetse-infested area (red cells), according to the baseline entomological data collection of the tsetse eradication campaign in the Niayes of Senegal.
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Fstat 2.9.3.2 (updated from [27]). However, more than two levels (i.e., individuals, sub-populations and total) may exist. This is the case for the different sub-samples belonging to the Niayes region when to be compared to tsetse from Missira. HierFstat version 0.03–2 [20] is a package for the statistical software R [29]. This package computes hierarchical F-statistics from any number of hierarchical levels [20]. A user-friendly description of the software is presented elsewhere [30].

The significance of the F-statistics was tested by randomization (10000 permutations in each case). The significance of \( F_{st} \) was tested by randomizing alleles between individuals within sub-samples. The significance of \( F_{st} \) was tested by randomizing individuals among sub-samples. These tests were performed with Fstat. The significance of \( F_{sr} \), i.e., the homozygosity due to subdivision into sub-samples within the Niayes region, was tested by randomising individuals among subsamples of the Niayes. The significance of \( F_{st} \), which measures the relative homozygosity due to the geographical separation between the Niayes region and Missira was tested by randomizing subsamples (with all individuals contained) between the two regions. These two last tests were performed with HierFstat. For \( F_{st} \) the statistic used was directly the \( f \) (unbiased estimator of \( F_{st} \)). For other differentiation measures (\( F_{st} \), \( F_{sr} \), \( F_{st} \)), the statistic used was the maximum likelihood ratio \( G \) [28,31].

Non random association of alleles within individuals (\( F_{is} > 0 \)) may be due to the presence of null alleles. We used Micro-Checker 2.2.3 [32] to detect null alleles and estimate their frequency \( p_n \) at each locus according to Brookfield’s second method [33]. For X-linked loci, males were not included in such analyses and null frequency was also directly estimated by the proportion of missing genotypes. The global expected frequency of blanks (null homozygotes) was then compared to the observed one with an exact unilateral [H1: there are less blanks than expected] binomial test with R.

Linkage disequilibrium between pairs of loci was tested using Fstat by randomising loci combinations (free recombination) across loci with a G-based statistics permitting a global test across sub-samples for each pair of loci. Because there are as many tests as locus pairs tested, we used the exact binomial test to check if the proportion of tests found significant at the 5\% level was significantly above 0.05 with the “Test alpha” and look for \( k^* \) option of Multitests V.1.2 [34]. To optimise power we disregarded loci displaying one allele at a frequency above 0.9, which was the case of locus XB110.

Some loci are X-linked. For \( F_{is} \) based analyses males were coded as missing data at these loci, otherwise these loci were coded as homozygous (differentiation and LD based analyses).

In order to visualize the partition in genetic differentiation among all samples, a NJTree dendrogram was constructed with the software MEGA 3.1 [35] from a chord distance matrix (noted \( D_{Chord} \)) [36], as recommended by [37], and computed using Genetix V4 [38].

**Effective population sizes.** Effective population sizes were computed with three methods. Estim 1.2 [39] uses the connection between migration and effective population size with heterozygosity and linkage disequilibrium between loci. Linkage disequilibrium is indeed connected to effective population size, a property exploited by the method from Bartley et al. [40], which is in fact coming from Hill [41] and modified by Waples [42]. From equation 2 of [40], modified as in NeEstimator Help file [43], allelic correlations \( \rho \) can be estimated as

\[
r = D \sqrt{p(1-p)q(1-q)},
\]

where \( p \) and \( q \) are frequencies of allele \( A \) at locus 1 and allele \( B \) at locus 2, respectively and \( D \) is the so called (e.g. [40,44]) Burrow’s composite measure of disequilibrium [45]. The \( r \)-values across all pairs of alleles are averaged to yield a single \( r \) for each pair of loci. Finally, an arithmetic mean of the \( r \) values for all pairs of loci is used to obtain a single correlation coefficient and to obtain an \( F_{st} \) estimate using the equation \( N_e = 1/3(r^2 - 1/S) \) where \( S \) is the harmonic mean of the sample sizes of each pairwise comparison between loci [40,46].

Males were coded homozygous for X-linked loci, which should not be a problem for a composite based linkage disequilibrium measure. The method was implemented with NeEstimator [43]. Heterozygote excess method from Pudovkin et al. [47] (see also [48]) corrected by Balloux [49], uses the fact that, in dioecious (or self incompatible) populations, alleles from females can only combine with alleles contained in males and a heterozygote excess is expected as compared to Hardy-Weinberg expectations, and this excess is proportional to the effective population size. This method was implemented using Weir and Cockerham estimator of \( F_{st} \) in the equation \( N_e = 1/(-2F_{st}) - F_{st}/(1 + F_{st}) \) [49] and was only applicable in subsamples with heterozygote excess.

For intra locus based methods (Estim and Balloux’s methods), loci showing evidence of stuttering and null alleles were removed.

**Bottleneck detection.** Signatures of bottleneck events were investigated by comparing the expected heterozygosity for a sample \( (H_e) \) with the heterozygosity that would be expected for a sample taken in a population at mutation/drift equilibrium with the same size and allele number \( (H_0) \). As allele number decreases faster than heterozygosity, a bottleneck is signed by \( H_e > H_0 \) in subsequent generations [50]. This analysis was performed with Bottleneck v1.2 software [51] assuming that mutations of microsatellite loci followed either an IAM (infinite allele model), a SMM (stepwise mutation model), or a TPM (two phase model), in the last case of which we assumed that 70\% of mutations consist of one step and 30\% consist of multistep change with a variance of 30 (default values). Tests were performed using unilateral Wilcoxon tests as recommended [50].

The method of bottleneck detection described by Cornuet and Luikart [50] allows a rough estimate of the effective population size right after the bottleneck event \( (N_{eb}) \). From the Figure 3A of [50], with 10 loci and mean sample sizes of 10–30 individuals, a bottleneck can be detected if it occurred between \( t_2N_{eb} \) and \( t_2N_{eb} \) generations before sampling with \( t_1 = 0.1 \) and \( t_2 = 2.5 \).

The difference in genetic diversity, as measured by \( H_t \) (Nei’s unbiased estimator [52]), between the Niayes and Missira was evaluated with a bilateral Wilcoxon signed rank test for paired data. The different microsatellite loci were used as the pairing factor. This test was undertaken with R.

The Bonferroni procedure [53] was used each time multiple testing was done and individual tests significance required.

User-friendly descriptions of most of the tests and procedures used in the genetic data analyses can be found in a recent review [54].

**Mitochondrial DNA markers.**

A portion of the 5’ end of the mitochondrial gene COI was amplified, purified and sequenced using the primers CI-J-2195 TTGATTTTTGGCTATCCAGAAGT [55] and CULR TGAAGCTAATCCGACTAATC using the same conditions reported by [56]. The following statistics were calculated using DNAsp version 4.50.3 [57]: \( Hd \) Haplotype diversity, was calculated using equations 8.4 and 8.12 in [58], \( P_h \), the nucleotide diversity, which is the average number of nucleotide differences per site between two sequences, and its sampling variance was calculated using equations 10.5–10.7 in [58], \( K \), the average number of nucleotide differences and the total variance of \( K \) is (sampling plus stochastic), assuming no recombination were calculated using equations from [59]. \( F_{st} \) was calculated according
to equation 3 in [60], $H_{ST}$ was calculated according to equation 2–4, and $K_{ST}$ according to equations 7–11 in [60]. $H_{ST}$ is a haplotype frequency based genetic differentiation statistic that does not take into account the number of differences separating different haplotypes. $H_{ST} = 1 - (H_r / H_t)$, where $H_r$ is the weighted average of subpopulation genetic diversities and $H_t$ is the estimated haplotype diversity of the total population. $K_{ST} = 1 - (K_r / K_t)$, where $K_r$ is a weighted average of the log corrected average number of sequence difference in the populations being compared, and $K_t$ is the average number of difference between sequences. A permutation test, in which haplotypes or sequences were randomly assigned to the different localities 10000 times, was used to test the significance of $H_{ST}$ and $K_{ST}$ [60]. The average number of nucleotide difference (equation A3, [61]) and its variance (sampling plus stochastic) were also calculated using DNAsp version 4.50.3. Haplotype trees for 738 nucleotides of COI from G. p. gambiensis (data from a total of 148 individuals in this study) were generated using the algorithm of [62]. The TCS 1.21 programme was used to estimate the haplotype tree, with the connection limit (probability of parsimony) at 95% [63]. The maximum number of connection steps at 95% was 11.

Geometric morphometrics
In total, the number of analysed wings was 20, 21, 18, 34 and 18 for Diczasaw Peul, Dakar Hann, Sebkotan, Missira and Pout respectively. These analysed individuals were all also analysed by the molecular markers.

Wings were dry-mounted between two microscope slides and scanned with a scanner. From this picture, 10 landmarks defined by vein intersections were recorded as previously described [64]. Each landmark has X and Y coordinates, and the 10 LM defined per wing represent a polygon. After scaling, translating and rotating all these polygons so that they can be compared, data were subjected to generalized Procrustes analysis (GPA) [65,66] allowing to implement shape variables, here represented by 16 “partial warps” (PW) (including uniform component of shape). These PW were used to conduct a discriminant analysis to allow for individual reclassification based on Mahalanobis distances (noted $D_{MH}$), which were calculated between populations. The statistical significance of Mahalanobis distances was estimated by 1,000-runs permutation tests [67]. A re-classification score was computed where individuals are assigned to each group and the percentage of good classification was then calculated.

Evaluating correlation between genetic distances and morphometric distance matrices
To evaluate the correlation between distance matrices we undertook three Mantel tests [68] with the “Mantelize it” option of Fstat 2.9.4 [27] between $D_{CS-SRE}$ and $K_{ST}$, between $D_{CS-SRE}$ and $D_{AB}$, and between $K_{ST}$ and $D_{AB}$.

Results
Microsatellite loci
Within subsamples analyses. The per-locus number of alleles was 3.4, 3.7, 3.9 and 4 for Diczasaw Peul, Dakar Hann, Sebkotan and Pout respectively (all coming from the Niayes), but it was 8.7 for Missira. The Wilcoxon test confirmed that mean genetic diversity in the Niayes ($H_t = 0.5$) was lower than the one found in Missira ($H_t = 0.7$) ($P$-value = 0.0059).

There was a global and significant homozygote excess over all loci ($F_{IS} = 0.17$, $P$-value = 0.0001). Figure 2 shows that this is mainly due to certain loci (i.e. pGp1, pGp24, A10 and XB110). This strongly suggests locus specific technical problems. Micro-
An analysis of the shape of the wings was conducted by using Procustes analysis to compare wing shape among the five different populations. All Mahalanobis distances between populations were significant except between Pout and Sebikotan. This is illustrated by reclassification scores which were over 70% indicating differentiation between populations (see Table 3). Missira sample showed the best one (85%) confirming this sample was easily distinguished from the others.

**Morphometrics**

The correlation between the genetic distances and morphometric distances were all strong and significant (see Figure 5). The strong correlation between microsatellites and COI showed the best one (85%) confirming this sample was easily distinguished from the others.

**Correlation between distance matrices**

The correlation between the genetic distances and morphometric distances were all strong and significant (see Figure 5). The strong correlation between microsatellites and COI showed the best one (85%) confirming this sample was easily distinguished from the others.

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**Figure 2.** $F_{IS}$ across loci and overall loci. For each locus 95% confidence intervals (CI) were obtained by jacknives over populations. Overall loci (All), CI were obtained by bootstrap over loci. Deviations from panmixia are given as corresponding $P$-values between brackets.

**Figure 3.** Unrooted Cavalli-Sforza and Edward’s chord distance based Neighbour-Joining-Tree. This tree shows the respective genetic distribution of the five *G. palpalis gambiensis* tsetse samples from Senegal.

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![Figure 2](image-url)

![Figure 3](image-url)
distance matrices was mainly due to the fact that there are two sets of points: those from the Niayes sites and those differentiating the Niayes from Missira (very strong distances for both markers). For the correlation between genetic and morphometric data, Figure 5 shows that around 50% of the variance in Mahalanobis distance can be explained by genetics, meaning that the other 50% are probably explained by environmental parameters.

Discussion

The results of the genetic and morphometric analyses indicated limited gene flow between the *G. p. gambiensis* population of the Niayes and those of the main tsetse belt in the south-eastern part of Senegal. Using three different kinds of markers, i.e. microsatellite DNA, mitochondrial DNA and geometric morphometrics, the data led to the conclusion that the *G. p. gambiensis* population from the Niayes can be considered isolated with very little risk of re-invasion should the population eventually be eradicated. These data corroborate the results of the entomological baseline data collection and observations in the field indicating the absence of *G. p. gambiensis* in the 120 km long area between Missira and the Niayes (JB, BS unpublished results.). On this basis, eradication of *G. p. gambiensis* from the Niayes can therefore be recommended as an appropriate control strategy. The data from this study, which was part of a comprehensive baseline data collection effort, confirmed that eradication can be recommended as an appropriate control strategy, and as such the study greatly assisted in the decision making on which strategy to select. Although the isolation of the target population is not an absolute prerequisite for AW-IPM, tackling a continuous pest populations is actually more complex, requiring more resources and a long-term, regional commitment [for details, see [10,11,14].

Looking at $F_{ST}$ values reported in previous studies on *G. p. gambiensis*, the one observed in the present work between Missira and the Niayes populations was ten times higher than those observed along 260 km on the Mouhoun river in Burkina Faso [17], and two times higher than the values observed in Guinea between Loos islands and the continent [64]. These values are of the same order of magnitude as those observed between the two different taxa, *G. p. gambiensis* and *G. pallalis pallalis* (DK, PS, unpublished data). It is also noteworthy that some of the microsatellite loci used on the individuals in this study amplified poorly, a behaviour not recorded in earlier studies of *G. p. gambiensis* [17,19,64]. This may be an additional argument for genetic divergence, since it is known that in different taxa polymorphisms in sequences flanking the microsatellite may occur [69] leading then to mismatches in the primer binding sites. If gene flow between the sites occurs then shared haplotypes of the mitochondrial gene COI would be expected. In fact, we observed no haplotypes shared between Missira and the Niayes region. Future work should include more detailed examination of

**Table 1. Population differentiation statistics for mitochondrial cytochrome oxidase 1 (COI) sequences (738 sites in alignment, no gaps).**

| Population | Population | $Hs$ | $Fst$ | $Kst^*$ | $Hst$ |
|------------|------------|------|-------|--------|-------|
| Missira    | Sebkotan   | 0.431| 0.88  | 0.642  | 0.405 |
| Missira    | Diacsaw Peul| 0.415| 0.883 | 0.646  | 0.426 |
| Missira    | Dakar Hann | 0.499| 0.876 | 0.607  | 0.350 |
| Missira    | Pout       | 0.611| 0.865 | 0.561  | 0.253 |
| Sebkotan   | Diacsaw Peul| 0.032| 0.000 | -0.002 | -0.002|
| Sebkotan   | Dakar Hann | 0.099| -0.02 | -0.007 | -0.007|
| Sebkotan   | Pout       | 0.200| 0.137 | 0.087  | 0.087 |
| Diacsaw Peul| Dakar Hann| 0.067| 0.038 | 0.022  | 0.025 |
| Diacsaw Peul| Pout      | 0.171| 0.208 | 0.134  | (0.05>P>0.01) |
| Dakar Hann | Pout       | 0.256| 0.062 | 0.035  | 0.035 |

Bold type indicates significant tests ($P<0.001$) after sequential Bonferroni correction for multiple testing.

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**Figure 4. Haplotype trees for 738 nucleotides of COI from *G. p. gambiensis*.** Nodes are labelled with the name of one of the individuals having a haplotype at that node. All the Niayes haplotypes are included in the nodes labelled H1 and H2. Node size is proportional to the number of haplotypes at that node. Abbreviations: H: Dakar Hann, M: Missira. The numbers on the branches indicate the position of the nucleotide in the alignment which is mutated.

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potential cryptic species within the distribution area of G. p. gambiensis since such cryptic species have been suspected and shown to exist in closely related taxa [70,71].

In case the Government of Senegal decides to integrate the sterile insect technique (SIT) as part of the AW-IPM (area-wide integrated pest management) approach, it would be advisable that prior to the release of sterile males, experiments be conducted to assess mating compatibility between the G. p. gambiensis from the Niayes and those which should be released if they do not originate from the same area (e.g. from Burkina Faso). Although previous studies with G. p. gambiensis originating from Mali and Burkina Faso revealed no mating barriers between these populations (G. Mutika, personal communication), nor did another study between the same area (e.g. from Burkina Faso). Although previous studies with G. p. gambiensis originating from Mali and Burkina Faso revealed no mating barriers between these populations (G. Mutika, personal communication), nor did another study between the same area (e.g. from Burkina Faso). Although previous studies with G. p. gambiensis originating from Mali and Burkina Faso revealed no mating barriers between these populations (G. Mutika, personal communication), nor did another study between the same area (e.g. from Burkina Faso). Although previous studies with G. p. gambiensis originating from Mali and Burkina Faso revealed no mating barriers between these populations (G. Mutika, personal communication), nor did another study between the same area (e.g. from Burkina Faso).

The second question that has relevance for a future AW-IPM programme was to know whether within the Niayes region, the four populations showed any genetic differentiation, or if they constituted a single, panmictic (i.e random mating) population. Low haplotype diversity of the populations of this area is in agreement with a previous Single Strand Conformation Polymorphism-based study on mitochondrial DNA haplotypes in the Niayes region of Senegal [75]. The Niayes population is probably a remnant population, possibly of small size and therefore likely to lose rare haplotypes by genetic drift more rapidly than larger populations. Many ancestral G. p. gambiensis haplotypes have probably been lost from the Niayes region, leaving just two haplotypes remaining today. The low diversity was also observed at microsatellite loci with lower genetic diversity than in Missira. The three different markers used in this study generally showed good agreement in the differentiations observed, as can be seen by the high and significant correlations coefficients computed between them.

The results suggest that the samples from Diacsaw Peul, Sebikotane and Pout, although showing some genetic differentiation, are not completely isolated from each other. This is also consistent with ecological data since it appears that the maximum distance between the forest patches of this area is less than 2km (data not shown.).

To conclude, the use of genetic and morphometric markers has been instrumental in the decision-making process of selecting and developing of an appropriate intervention strategy to create a sustainably zone free of G. p. gambiensis and Trypanosomosis in the Niayes region of Senegal. In the near future, it should be encouraged to carry out such studies prior to the selection of target areas or the choice of control strategies, and these should be part of the overall collection of baseline data (see ref [76] for recent review). In addition, the results obtained here suggest that efforts should be made to look for other genetic discontinuities in G. palpalis s.l. distribution that may be indicative of the presence of cryptic species.

### Table 2. Diversity statistics for the studied populations based on COI sequences.

| Population | Number of individuals sequenced | Number of Haplotypes | Polymorphic Sites | H[D]. | PI | K (variance) |
|------------|---------------------------------|----------------------|-------------------|-------|----|-------------|
| Missira    | 34                              | 9                    | 10                | 0.777 (3.87 x 10^-3) | 0.00271 (2 x 10^-7) | 1.996 (1.333) |
| Sebikotan  | 32                              | 2                    | 1                 | 0.063 (3.33 x 10^-3) | 0.00008 (0 x 10^-7) | 0.063 (0.019) |
| Diacsaw Peul | 30                              | 1                    | 0                 | 0.000 (0.0000) | 0.00000 (0 x 10^-7) | 0.000 (0.000) |
| Dakar Hann | 27                              | 2                    | 1                 | 0.142 (7.43 x 10^-3) | 0.00019 (0 x 10^-7) | 0.142 (0.045) |
| Pout       | 25                              | 2                    | 1                 | 0.380 (8.33 x 10^-3) | 0.00051 (0 x 10^-7) | 0.380 (0.139) |
| Total      | 148                             | 11                   | 15                | 0.483 (3.39 x 10^-7) | 0.00441 (2 x 10^-7) | 3.257 (2.849) |

### Table 3. Geometric morphometrics: reclassification scores of individuals in their population of origin.

| Population       | Proportion of individuals correctly reclassified in their population (%) |
|------------------|-------------------------------------------------------------------------|
| Diacsaw Peul     | 15/20 (75)                                                             |
| Dakar Hann       | 16/21 (76)                                                             |
| Sebikotan        | 14/18 (77)                                                             |
| Missira          | 29/34 (85)                                                             |
| Pout             | 13/18 (72)                                                             |

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Figure 5. Mantel correlation tests ($P$-values) between the different markers used in this study. Mantel correlation tests and percentages of variance explained ($R^2$) between (a) Cavalli-Sforza and Edwards ($D_{C-S&E}$) computed from microsatellite data and Kst* computed from COI mitochondrial data, (b) $D_{C-S&E}$ and Mahalanobis distance ($D_M$) computed from morphometric data, and (c) Kst* and $D_M$ for tsetse flies from the five different samples from Senegal (10 distances).

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Supporting Information

Alternative Language Abstract S1  Translation of the abstract into French by PS.
Found at: doi:10.1371/journal.pntd.0000692.s001 (0.03 MB DOC)

Table S1  Effective population size estimates according to the different methods described.
Found at: doi:10.1371/journal.pntd.0000692.s002 (0.02 MB XLS)

Table S2  Probabilities of detecting bottleneck in each population according to microsatellite mutation model.
Found at: doi:10.1371/journal.pntd.0000692.s003 (0.02 MB XLS)

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