Population Structuring of *Glossina palpalis gambiensis* (Diptera: Glossinidae) According to Landscape Fragmentation in the Mouhoun River, Burkina Faso

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ABSTRACT The impact of landscape fragmentation due to human and climatic mediated factors on the structure of a population of *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) was investigated in the Mouhoun river basin, Burkina Faso. Allele frequencies at five microsatellite loci, and metric properties based on 11 wing landmarks, were compared between four populations. The populations originated from the Mouhoun river and one of its tributaries. The average distance between samples was 72 km with the two most widely spaced populations being 216 km apart. The sampling points traversed an ecological cline in terms of rainfall and riverine forest ecotype, along a river enlarging from downstream to upstream and oriented south to north. Microsatellite DNA comparison demonstrated structuring between the populations, but not complete isolation, with an overall $F_{st} = 0.012$ ($P < 0.001$). Wing geometry revealed significant centroid size and shape differences between populations, especially between the two most distant populations. There was no significant correlation between gene flow and geographic distance at this scale, but there was a positive correlation in females between metric distances (wing shape differences) and geographic distances that might be attributed to the cline of environmental conditions. The impact of the fragmentation of riparian landscapes on tsetse population structure is discussed in the context of control campaigns currently promoted by Pan African Tsetse and Trypanosomosis Eradication Campaign.

KEY WORDS *Glossina palpalis gambiensis*, microsatellite DNA, geometric morphometrics, isolation, Burkina Faso

Control of tsetse can be achieved through a variety of techniques, including traps, insecticide-impregnated targets, live-baits, sequential aerial spraying, and sterile male release (Cuisance et al. 2003). Generally, however, the tsetse populations tend to recover due to either flies surviving the initial interventions or to migrant flies coming from untreated regions, or both (Cuisance et al. 1984, Politzar and Cuisance 1984, de La Rocque et al. 2005). To choose between elimination or sustainable control of a tsetse population, knowledge of their population structure is of key importance. In isolated populations, elimination may be feasible, as demonstrated for *Glossina austeni* Newstead in Unguja Island of Zanzibar in 1997 (Vreysen et al. 2000). But, for most mainland populations of tsetse, the geographical limits of target tsetse populations are less easily definable. Application of population genetics techniques can help in understanding and quantifying gene flows between subpopulations as an indirect measure of dispersal (Gooding and Krafsur 2005), and it can assist in choice of control strategies, development of artificial barriers, or both (Politzar and Cuisance 1983).

In Burkina Faso, the main vectors of African trypanosomoses are tsetse flies of the *palpalis* group (subgenus *Nemorhina*) that thrive in vegetation along rivers (Challier and Gouteux 1980, Bouyer et al. 2005, Bouyer et al. 2006). *G. morsitans submorsitans*, the only tsetse of the *morsitans* group (subgenus *Glossina*) in the country, is now restricted to the conserved areas (wild fauna reserves) of the southern border with Ivory Coast, Ghana, Togo, and Benin. In the Mouhoun river basin, riparian species seem to be more resilient to land-use changes, due to their ability to adapt to peridomestic situations and to their linear habitat (Cuisance et al. 1985, Bouyer et al. 2007), which allows

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them to cross easily between favorable patches, riverine forests acting as genetic corridors.

In Burkina Faso, initial studies have demonstrated strong structuring between *G. palpalis* populations in fragmented landscapes of the agropastoral area of Sidéradougou (Solano et al., 1997, Solano et al. 2000). Recent work revealed that the distribution and abundance of riverine flies is associated with the type and disturbance level of the gallery forest (Bouyer et al. 2005), which allowed mapping of the risk of African animal trypanosomoses (AAT) risk along a 234-km-long river section of the Mouhoun river (Bouyer et al. 2006). Tsetse demonstrated a patchy distribution with the highest transmission risk located at the border of the conserved forests. The understanding of the relations and particularly the migration of individuals between the favorable ecological patches is of key importance to design a control campaign.

We used genetic variation at microsatellite DNA loci, together with phenetic variation in wing geometry to examine the population structure of *G. p. gambiensis.* These two approaches have demonstrated concordance in an isolated population of *G. p. gambiensis* in Guinea (Camara et al. 2006). The objective of the current study was to assess the impact of landscape fragmentation within the Mouhoun river basin on the structuring of tsetse populations. Currently, a control campaign is to be launched in this area by the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), which will use the present results to identify successive blocks that should be treated sequentially (Fond Africain de Développement 2004).

Materials and Methods

Study Area. The Mouhoun river basin in Burkina Faso is experiencing landscape fragmentation through human-driven changes of peririverine landscapes (cropping and cattle grazing). Crops (especially cotton, *Gossypium hirsutum* L.) located 500 m beside the river course cause a disturbance of the gallery forests, with a negative impact on tsetse densities (Bouyer et al. 2005, Bouyer et al. 2006). The intervening undisturbed areas still harbor high densities of tsetse, but the exchanges between favorable habitat patches are not known.

In the present article, the part of the Mouhoun river under study is called the Western branch, extending from the Dinderesso Forest (4° 26′ W, 11° 13′ N), a protected area located at the southwestern extremity of the Kou river (a tributary of the Mouhoun), to the Sourou dam (3° 26′ W, 12° 44′ N) at the extreme north of the Mouhoun river. Four locations roughly oriented from south to north were sampled, with between-samples distances of 74, 61, and 81 km upstream to downstream, totalling 216 km between the first and the fourth location (Fig. 1). The four sample sites are located along an ecological cline caused by enlargement of the river course, ranging from a Guinean gallery forest next to the source for sample A, where *G. palpalis gambiensis* predominates over *G. tachinoides* Westwood, through a Sudano-Guinean gallery forest for samples B and C, where both species are found in similar densities, to a gallery becoming of Sudanese ecotype in the northern part for sample D, located at the northern edge of the distribution area of this species which scarcely occurs downstream, where *G. tachinoides* becomes the predominant species (Bouyer et al. 2005). In addition, there is also variation in rainfall between the locations, because rainfall of sample A (the most southern) is ≈1,100 mm/yr, but it decreases toward the north to reach ≈800 mm/yr in sample D location. From dispersal models, it can be assumed that no tsetse can fly actively from one population to the other ($P < 10^{-19}$, for a diffusion model using a diffusion coefficient of 0.500 $km^2\ d^{-1}$ and a daily mortality rate of 0.03 (Bouyer et al. 2007). Mark–recapture studies conducted in the first location estimated a mean displacement of 2 km corresponding to a mean lifespan of 53 d and a density of 3,599 *G. p. gambiensis* (95% CIs, 2570–4628) for sample A (Bouyer 2006).

Entomological Surveys. Entomological surveys were conducted during the 2002 hot dry season (April and May for the western branch) (Bouyer et al. 2005) by using standardized biconical traps (Challier and Laveissière 1973) 100–150 m apart, operated from 0900 to 1630 hours. The 348 trap locations were recorded using global positioning system. Tsetse flies were recorded by species and by trap (apparent density per trap per day or ADT) and dissected in the field. From each dissected tsetse, the wings were removed, dry-mounted between a labeled slide and coverglass (three pairs of wings by slide), and sealed with Canada balsam. Three legs were removed and put in individual, labeled, dry Eppendorf tubes.

Morphometrics. In some individuals one wing was damaged and only one wing could be analyzed, so that from samples A to D, respectively, 52 (22 pairs and eight single wings), 52 (22 pairs and eight single wings), 50 (22 pairs and 6 single wings), and 56 wings (20 pairs and 16 single wings) were analyzed in female *G. p. gambiensis,* and 50 (21 pairs and eight single wings), 53 (21 pairs and 11 single wings), 53 (20 pairs and 13 single wings), and 51 wings (19 pairs and 13 single wings) were analyzed in males. Wings were photographed with a microscopic digital camera (MoticR). From this picture, 11 landmarks defined by vein intersections were recorded as described by Camara et al. (2006). Separately for males and females, the data were subjected to generalized Procrustes analysis (GPA) (Rohlf 1990, 1996).

Centroid size (Bookstein 1991) and its variance were recorded. Shape variables, here represented by 18 "partial warps" (PW) (including uniform component of shape), were used for geographic comparisons and classification. Unweighted pair-group method with arithmetic average trees were derived from Euclidian distances among average forms corresponding to each locality. The significance of each pairwise distance was assessed by simple permutation tests (1,000 runs).

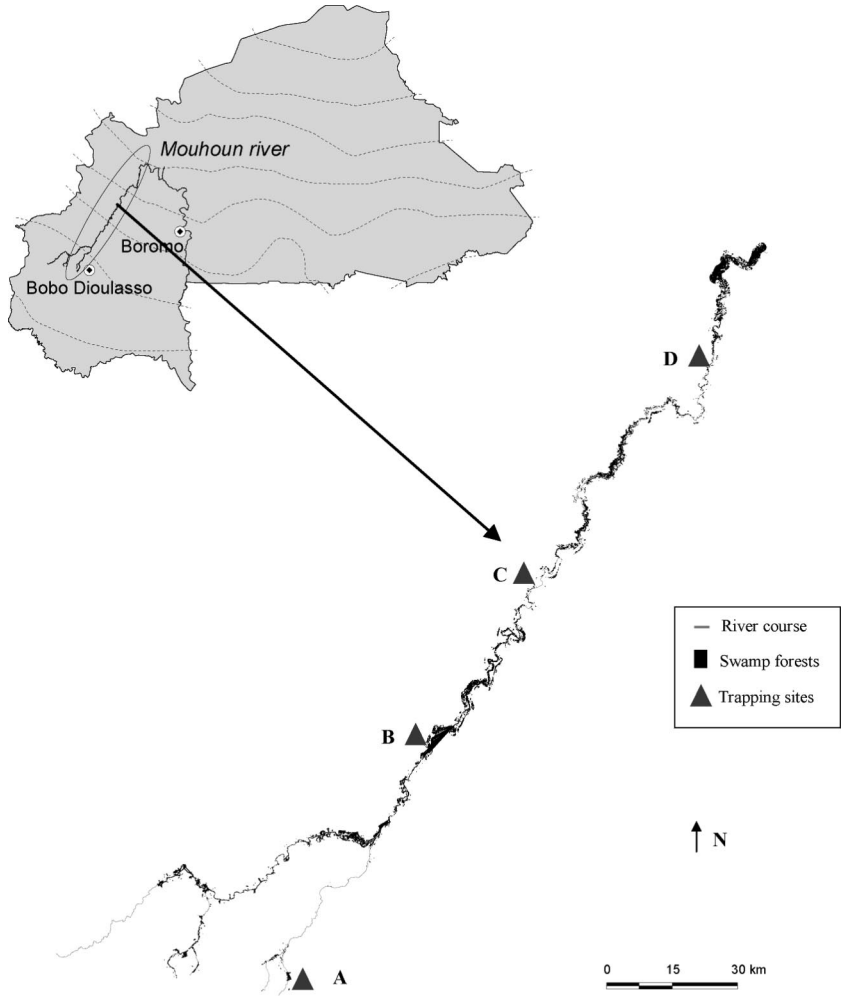

Fig. 1. Geographic location of the study area and distribution of the "protective" swamp forests in 1,000-m buffers around the water course. A, B, C, and D are the four sample locations.

A discriminant analysis was also conducted on PW to allow for individual reclassification based on Mahalanobis distances. The percentage of correctly assigned individuals was computed for each locality. The residual allometry was estimated by multivariate regression of PW on (centroid) size, and statistical significance estimated by 1,000 permutations (Good 2000). To estimate the contribution of size variation to the geographic distinction provided by the discriminant functions, each of these functions was regressed on size variation. The common allometric model hypothesis was explored by a multiple analysis of covariance with significance based on Wilks statistics (Dujardin et al. 2007). Metric disparity (MD) was computed and compared between localities or sexes as described by Zelditch et al. (2004).

Microsatellite Loci. DNA extraction followed the protocol described by Walsh et al. (1991). To each tube containing the legs of the tsetse, which were crushed, 200 μl of 5% Chelex chelating resin was added (Walsh et al. 1991, Solano et al. 2000). After incubation at 56°C for 1 h, DNA was denatured at 95°C for 30 min. The tubes were then centrifuged at 12,000 $\times$ *g* for two minutes and frozen for later analysis.

A total of five microsatellite loci was used : B104, C102 (kindly given by A. Robinson, Entomology Unit, Food and Agricultural Organization of the United Nations/International Atomic Energy Agency [FAO/IAEA], Agriculture and Biotechnology Laboratory, Seibersdorf, Austria); pgp13, pgp11 (Luna et al. 2001), and CAG133 (Baker and Krafsur 2001). C102 and CAG133 have trinucleotide repeats whereas the others are dinucleotides.

The polymerase chain reactions (PCRs) were carried out in a thermocycler (MJ Research, Cambridge, United Kingdom) in a 10-μl final volume, using 1 μl of the supernatant from the extraction step. After PCR amplification, allele bands were resolved on a 4300 DNA Analysis System from LI-COR (Lincoln, NE) after migration in 96-lane reloadable (3X) 6.5% denaturing polyacrylamide gels. This method provides for multiplexing by the use of two infrared dyes (IRDye),

separated by 100 nm (700 and 800 nm), and read by a two-channel detection system that uses two separate lasers and detectors to eliminate errors due to fluorescence overlap. To determine the different allele sizes, a large panel of ≈30 size markers was used. These size markers had been previously generated by cloning alleles from individual tsetse flies into pGEM-T Easy Vector (Promega, Madison, WI). Three clones of each allele were sequenced using the T7 primer and the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Sequences were analyzed on an Applied Biosystems 310 automatic DNA sequencer, and the exact size of each cloned allele was determined. PCR products from these cloned alleles were run in the same acrylamide gel as the samples, allowing the allele size of the samples to be determined accurately. Genotyping was achieved twice (two independent readers) and a consensus was achieved for each individual (when consensus was not possible, the individual was removed from the analysis).

Pedigree Analysis of Microsatellite Loci. Controlled crosses were performed with nine female/male pairs of insectary-reared *G. p. gambiensis,* which provided three to 11 F1 individuals each. Pedigree analysis was used to verify the X-chromosome location of microsatellite loci. It also was used to look for possible null alleles, but their frequencies could not be computed because of the low number of tsetse pairs and the low number of F1 offspring from each tsetse pair.

Microsatellite Data Analysis. In total, 120 *G. p. gambiensis* was used for the genetic analyses at microsatellite loci: 30 in sample A (the most upstream) with 13 females and 17 males, and 30 females from each of the three others samples.

For the total sample subdivided by the four localities, Wright's F_{is} (within-sample heterozygote deficiency, a measure of deviation from random mating; (Wright 1951) and F_{st} (measure of population differentiation) were estimated using Weir and Cockerham's unbiased estimators (f for F_{is}, θ for F_{st}) (Weir and Cockerham 1984). For random mating (within samples) or random distribution of individuals (between samples), F values are expected to be zero.

When F_{st} was measured, it was compared with F_{st} max $= 1 - H_s$ (Hedrick 1999, 2005), where H_s is the unbiased estimate of genetic diversity (Nei and Chesser 1983, de Meeüs et al. 2007).

The significance of F_{is} (deviation from random mating) at each locus, and over all loci, also was tested separately within each sample by using 10,000 permutations of alleles between individuals. Males were hemizygous at loci on the X-chromosome so that for these loci, measures of F_{is} and its significance were conducted only on females. The significance of F_{st} (population differentiation) was assessed using 10,000 permutations of genotypes among samples. To evaluate significance when multiple tests were performed, the sequential Bonferroni procedure was applied (Rice 1989).

Software. The H_s, F_{is}, and F_{st} estimators were calculated with FSTAT version 2.9.3 software (Goudet 1995). The Cavalli-Sforza and Edwards (1967) chord distances were computed by the GENETIX version four software package (Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France). Anatomical data collection and subsequent morphometric analyses used specific software developed by one of us (J.-P.D.) and are available under GPL license at http://www.mpl.ird.fr/morphometrics, namely, COO for the collection of landmarks, MOG for the generalized Procrustes analysis, PAD for the discriminant analysis and regression of discriminant functions on size, and COV for the remaining analyses: total residual allometry, test for a common allometric model and analyses on metric disparity. The comparisons of size and its variance made use of a new module (VAT) programmed by Harling Caro-Riano and J.-P.D.

Isolation by distance was tested using the ISOLDE program available in Genepop 3.1 (Raymond & Rousset 1995). The principle is to have a matrix consisting in $F_{st}/(1 - F_{st})$ (for microsatellite data), or Mahalanobis distances (for morphometry) plotted against geographic distances, calculated as the length of the river course between the sample sites, because the species disperse in a linear habitat. Spearman rank correlation coefficients were used to test hypotheses that morphometric/genetic distances were correlated with geographic distances

Results

Morphometrics. *Centroid Size and Its Variance.* Comparison of the centroid sizes and their variances between samples gave different results according to the sex considered.

In males, the centroid size (c.s.) of population B was smaller than the three others ($P < 0.05$), which were of similar sizes ($P > 0.05$), whereas its variance was not different between populations ($P > 0.05$).

In females, the c.s. of populations A and D were similar ($P > 0.05$) and were both higher than population C ($P < 0.05$). Only the c.s. of population D was significantly higher than population B. Centroid size variance was smallest in population B, significantly smaller than in populations A (which shows the greatest variance) and C ($P < 0.05$) but not than population D ($P > 0.05$).

Shape and Metric Disparity. The unweighted pair-group method with arithmetic average classification tree derived from Euclidian distances between average forms corresponding to each sex and locality (Fig. 2) indicated that populations A and D were the most distant.

In females, the metric disparity was similar in all populations ($P > 0.05$), whereas in males, population A was significantly less disparate than populations B and C (the most heterogeneous). Population D was significantly less polymorph than population C, but not than population B ($P > 0.05$).

The discriminant analysis conducted on the 18 partial warps provided satisfactory individual reclassification rates (Table 1), confirming the differences be-

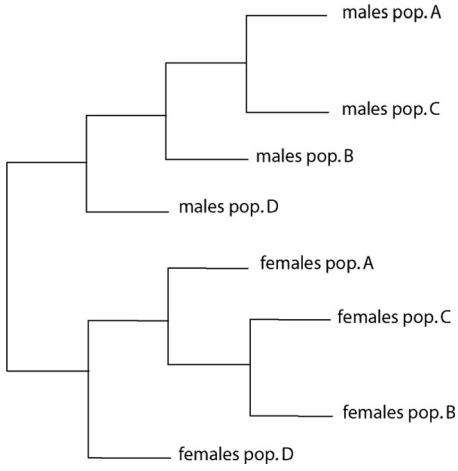

Fig. 2. Unweighted pair-group method with arithmetic average classification tree of the four samples along the western branch of the Mouhoun river, derived from Euclidian distances between average forms of the wings corresponding to each locality. All groups were found significantly different.

tween samples. The residual allometry was significant in females ($P < 0.001$) but not in males ($P = 0.646$), explaining why the unweighted pair-group method with arithmetic average tree was different between sexes: the female tree was not only based on shape variation but also on size differences. Moreover, in females, the hypothesis of a common allometric model was not supported ($P < 0.0001$; Wilks), although further comparisons indicated the common model did apply when population A was removed.

In summary, samples A and D showed numerous morphometric differences that did not support the idea of frequent exchanges of individuals between them. Population A had many differences compared with the others: the highest centroid size of females (together with sample D), the greatest variance of size, the smallest metric disparity, and a different growing axis.

Isolation by Distance. Mahalanobis distances derived from shape variation were plotted against geographic distances. Significant correlation was found in females ($P = 0.03$) and nearly so in males ($P = 0.12$), suggesting an isolation by distance model for metric variation (Fig. 3). When classified according to these distances, significantly higher proportions of each locality and sex were correctly assigned. Misclassified individuals were not distributed at random ($\chi^2 = 16.5102$, df = 6, $P = 0.01126$), but preferentially in the neighboring localities, again suggesting a process of isolation by distance.

Table 1. Individual reclassification rates obtained from a discriminant analysis conducted on the 18 partial warps (to be compared with a random reclassification rate of 12.5%)

Pop	% males	% females
A	50	32
B	43	51
C	20	42
D	54	66

Genetics. Pedigree analysis indicated that three of five microsatellite loci were located on the X-chromosome (B104, pgp11, and pgp13). For all loci, genotypes were in accordance with Mendelian segregation although ratios could not be tested due to small numbers of offspring.

From the 120 individuals analyzed, the number of alleles at each locus was 10, 11, 12, 4, and 6 for loci B104, pgp11, pGp13, C102, and Cag133, respectively.

Weir and Cockerham (1984) estimators indicated an overall F_{is} of 0.109 ($P < 0.001$) and an averaged F_{is} per population of −0.01, 0.17, 0.06, and 0.16, respectively, for samples 1–4, the second and fourth values being significant (Fig. 4). The F_{is} values were very variable between loci and samples and the presence of null alleles in our samples could not be rejected.

Overall F_{st} averaged +0.012, with $P < 0.001$, indicating genetic differentiation among the four populations. Isolation by distance was not found significant (data not shown).

Because H_s was 0.625, F_{st} max = 1 − H_s was 0.375. A standardized estimate of F_{st} would thus give $F_{st}' = F_{st}/F_{st}$ max = 0.032. Should this value have been close to 1, a complete lack of migrants would have been supported. The low F_{st}' value (0.032) rather suggests a significant impact of gene flow or a very recent isolation that would not yet be detectable.

Discussion

The aim of this study was to diagnose possible isolation of tsetse populations along a single hydrographical network. This question has important implications because the PATTEC national program in Burkina Faso aims at eliminating tsetse in this area by an area wide insect pest management strategy (Fond African de Développement 2004), and depending on the answer to this question, elimination strategies will vary very much: if there is no isolation, the control campaign must take all the hydrographical network as a whole, whereas if there is isolation, a sequential strategy may be undertaken.

As reported in other studies on tsetse using microsatellite DNA markers (Camara et al. 2006, Ouma et al., 2007), null alleles may be partly or totally responsible for the overall F_{is} positive value ($F_{is} = 0.109$). Other studies in *G. palpalis* showed that high F_{is} values could also be attributed to a Wahlund effect caused by the presence of individuals originating from different subpopulations in the same traps, in *G. p. gambiensis* (Solano et al. 2000) and in *G. p. palpalis* (Ravel et al. 2007).

In the present work, data from microsatellites and from wing geometry both converged to the idea of a structuring of *G. palpalis gambiensis* along the Western branch of the Mouhoun river. This was illustrated by the different wing growth axis of sample A, differences of centroid size and its variance, and also shape and its variance, between the four samples; and also by

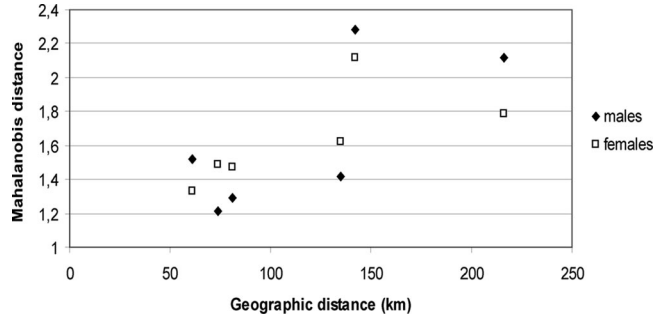

Fig. 3. Mahalanobis distances of female and male wings in samples A to D compared with geographic distances between their collection sites.

an overall genetic differentiation between the four localities.

However, the population structuring indicated by wing morphometry seemed more important than the slight (although significant) F_{st} value detected with microsatellites. That metric differences suggested isolation by distance in the female samples, whereas genetic results did not detect it, may be due to the geographic distance being superimposed to an "ecological distance" due to a double cline in both rainfall and riverine forest ecotype, from the south (sample A) to the north (sample D) (Morel 1978, Bouyer et al. 2005).

Metric properties are under the influence of both environmental and genetic factors. Environment typically acts primarily on size (Glasgow 1961), and then on shape, frequently as an allometric effect of size change (Dujardin and Le Pont 2004). In our four samples, the smallest males were not found in the same populations as the smallest females, nor the largest males with the largest females, indicating variable sexual dimorphism of size through the populations. The isolation by distance found in females was not significant in males, whereas the females' conformation was also under influence of size. So the apparent isolation by distance seems most likely due to the ecological cline.

The lack of evidence for a common allometric model among all females was attributable to population A, which suggested a further level of isolation between this population and the three others. However, allometry-free shape differences seemed to exist for males. Such differences among conspecific populations could result from adaptive or genetic causes rather than from environmental effects, unless local ecological conditions show huge differences (Dujardin and Le Pont 2004; Dujardin and Slice, 2007).

The morphometric data show structuring between populations. Genetic data are not incompatible with the persistence of gene flow between populations at this scale, or with a very recent isolation that would not yet be detectable using microsatellite markers. According to Falconer (1981), the first characters to show any change in case of recent isolation are continuous characters, such as morphometric characters. According to our working hypothesis, genetic markers open a window on a relatively remote evolutionary past, whereas the morphometric marker shows current and recent changes.

It is then essential to compare these results with the knowledge from dispersal models, ecology data ,and landscape analysis. Along this river section, the available dispersal models would predict an absence of migrants between the studied samples (Bouyer et al. 2007). However, from an ecological point of view, samples B, C, and D are not isolated from each other, because the riverine forests are still "conserved" to "half-disturbed" all along this section, allowing a continuous tsetse population (although variable in size) to occur between these three locations. It is thus not

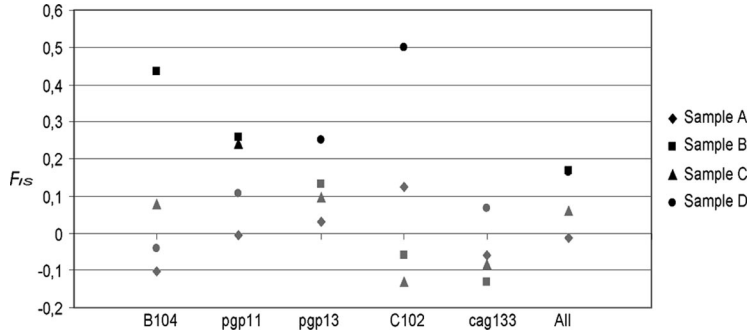

Fig. 4. Microsatellite markers: F_{is} per population at the five loci and for all loci (significant values in black, others in gray).

surprising that these populations still seem genetically connected, because there are intermediary populations between them. A one-dimensional model of isolation by distance corresponding to the values measured in the four samples (although not significant), would predict a sampled population size of 3,538 flies by site for a mean distance of 2 km between the adult birth places and their parent birth places, which is compatible with the measure obtained in sample A from mark–recapture trials (3,599 flies; 95% CI, 2,570–4,628). We could make the hypothesis that because migrants cannot pass directly from one population to another, their descendants evolve quickly from a morphological point of view under environmental pressures in the intermediary populations, maintaining the morphological differences between populations, but preventing genetic drift, then reducing genetic distances. The case of sample A is particular, because it is separated from the others by >50 km of disturbed riverine forest (this tributary crosses the Kou valley, which has intensive irrigated rice culture): there are almost no intermediary tsetse populations between populations A and B.

In the present situation, dispersal models and morphometric results converge in diagnosing an isolation of population A, although this is not confirmed by genetic results. This suggests either that population A has been recently isolated from the others (for example, due to increasing landscape fragmentation) and our molecular markers do not have sufficient resolution to detect this isolation; or there is as yet no complete isolation but there is a strong environmental component in the morphometric results. Further studies are needed, by sampling more (and possibly intermediary) populations and/or analyzing genetic distances at individual level, to better understand the structure of tsetse populations in the Mouhoun river basin and the relationship among morphometrics, genetics in tsetse, and environmental constraints.

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