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Abstract
The Tsetse fly. Glossina palltdipes. (Diptera: Glossinidae) is an important vector of animal trypanosomiasis It has also been implicated in the transmission of pathogens that cause human African trypanosomiasis. Understanding how G. palhdipes populations vary temporally is necessary for effective intervention. Temporal variation in allele frequencies at eight microsatelhte loci was assessed by sampling local populations of G. palltdtpes. Samplings were carried out in 2000, 200 I, and 2003 in the Lambwe Valley and Nguruman areas in Kenya. Six polymorphic loci were scored. Allele frequencies were homogenous between seasons. Genetic differentiation was higher among dry season samples ($F_{,}$ = 0.051, G" = 0.047) than wet season samples (F;r = 0.041, G" = 0.03 7). Differentiation between pooled dry season and pooled wet season samples did not differ (F,r = 0.008, G" = 0.004). Analysis of variance revealed no substantial genetic subdivision in seasons or years. It is concluded that G. pallidipes populations are more aggregated during the dry season, resulting in stronger measures of genetic differentiation when compared with wet seasons. However, season and time had no effect, indicating relative stability of G. pallidipes populations. Thus, strategies for suppression of G. pallidipes in the country should adopt measures that may not reduce effectiveness indifferent times of the year.

Keywords
Glossina, Trypanosomiasis control, vector allele frequencies variation

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THE INFLUENCE OF TEMPORAL AND SEASONAL CHANGES ON GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE TSETSE FLY, *GLOSSINA PALLIDIPES* IN KENYA

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ABSTRACT

The Tsetse fly, *Glossina pallidipes*, (Diptera: Glossinidae) is an important vector of animal trypanosomiasis It has also been implicated in the transmission of pathogens that cause human African trypanosomiasis. Understanding how *G. pallidipes* populations vary temporally is necessary for effective intervention. Temporal variation in allele frequencies at eight microsatellite loci was assessed by sampling local populations of *G. pallidipes*. Samplings were carried out in 2000, 2001, and 2003 in the Lambwe Valley and Nguruman areas in Kenya. Six polymorphic loci were scored. Allele frequencies were homogenous between seasons. Genetic differentiation was higher among dry season samples (F_{ST} = 0.051, G_{ST} = 0.047) than wet season samples (F_{ST} = 0.041, G_{ST} = 0.037). Differentiation between pooled dry season and pooled wet season samples did not differ (F_{ST} = 0.008, G_{ST} = 0.004). Analysis of variance revealed no substantial genetic subdivision in seasons or years. It is concluded that *G. pallidipes* populations are more aggregated during the dry season, resulting in stronger measures of genetic differentiation when compared with wet seasons. However, season and time had no effect, indicating relative stability of *G. pallidipes* populations. Thus, strategies for suppression of G. pallidipes in the country should adopt measures that may not reduce effectiveness in different times of the year.

Key words: Glossina, Trypanosomiasis control, vector allele frequencies variation

INTRODUCTION

Tsetse fly (*Glossina pallidipes* Austen) is one of the most important vectors of animal trypanosomiases in sub-Saharan Africa. Whereas the spatial genetic structure and genetic diversity of *G. pallidipes* populations has been widely investigated, temporal studies are lacking. Krafsur *et al.* (1997) detected surprisingly high levels of genetic differentiation among geographic populations from East and southern Africa at allozyme loci (F_{ST} = 0.238 ± 0.051). Essentially the same magnitude of differentiation was reported when variation was examined at mitochondrial DNA among eighteen *G. pallidipes* populations via single-strand conformational polymorphisms (Krafsur and Wohlford, 1999).

Comparative evaluation of variations at mitochondrial and microsatellite loci provided evidence that genetic distances were correlated with geographic distances between populations but not so at allozyme loci (Krafsur 2002). The foregoing reports are based on data from point samples in time, with an underlying assumption that genetic structure or diversity is relatively stable over time. However, this assumption does not always hold as allele frequencies drift over time (Waples, 1989 a, b). Temporal analyses can provide information about forces responsible for genetic changes over short time scales. Furthermore, assessing temporal changes in allele frequencies may indicate the occurrence of bottlenecks and provide estimates of change in effective population size (Richards and Leberg, 1996). From published ecological data on *G. pallidipes* it can be deduced that there is need to pay attention to temporal analyses of genetic data.

Ecological research employing baited traps has shown that *G. pallidipes* dispersion varies seasonally (Brightwell *et al.*, 1992; 1997). Mobility was much greater during the wet season than during the dry season (Brightwell *et al.*, 1992; Williams *et al.*, 1992), and was strongly correlated with rises in humidity and drops in maximum temperatures. What about relative populations densities? This raises the question concerning the extent to which *G. pallidipes* populations remain as independent entities and show local adaptations, especially during the dry season. Little is known about the

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adaptive genetic changes in *G. pallidipes* populations associated with time. Tsetse flies are long-lived and have low reproductive rates. Hence, they are likely to have small effective population sizes which are more subject to genetic drift, the chance change in gene frequencies that may occur from one generation to the next. The overall objective of the current study was to assess the effect of seasonal and temporal changes in the genetic structure of *G. pallidipes*. Specifically, the following null hypotheses were tested: 1) allele frequencies are homogeneous temporally; 2) there are no seasonal correlations in indices of population differentiation.

MATERIALS AND METHODS

Study sites and tsetse trapping

Lambwe Valley and Nguruman

Glossina pallidipes samples were obtained from the Ruma National Park in Lambwe Valley (henceforth referred to as Lambwe) and from the Nguruman area in Kenya. Genetic sampling of the study areas has been offered (Ouma *et al.*, 2006). The ecology of tsetse and trypanosomiasis in the two areas has been studied extensively (Turner, 1981; Turner and Brightwell, 1986; Brightwell *et al*., 1992, 1997). Tsetse flies were caught at geo-referenced sites using biconical traps (Challier and Laveissiere, 1973). Dry season samples were collected in November 2001 and July 2003. All wet season samples were collected in May 2003.

DNA extraction, amplification and GeneScan analysis

Total genomic DNA was isolated from ethanol-preserved *G. pallidipes* samples using a phenol/chloroform method as described by Krafsur and Wohlford (1999). The DNA was washed twice with ice-cold 70% ethanol, re-suspended in 100 µl of sterile double-distilled water. DNA samples were then stored at -20°C. Oligonucleotide primer sequences, polymerase chain reaction (PCR) conditions and GenBank accession numbers are reported elsewhere (Ouma *et al.*, 2008). Applied Biosystems (ABI) Prism 377 sequencer was used for electrophoresis of PCR products and size standard mixture. Genotype data was analyzed using *GeneScan* and *Genotyper* software.

Data analysis

Allelic frequencies were calculated for each sample and sampling date while deviations from Hardy-Weinberg expectations for each locus in each sample were determined by using exact tests (Guo and Thompson, 1992) and by tests assuming an alternative hypothesis of heterozygote deficiency (Rousset and Raymond, 1995). Genotypic linkage disequilibrium was assessed for each sample and sampling date. The program GENEPOP 3.4 (Raymond and Rousset, 1995) was used for these tests. Hypothesis of homogeneity of allele frequencies among seasons was tested using one-way Kruskal-Wallis non-parametric analysis of variance test and the means separated using Student's two-sample *t*-test (Snedecor and Cochran, 1989).

Genetic differentiation among all samples and between pairs of samples was estimated with three differentiation indices. First, F_{ST} was estimated according to Weir and Cockerham (1984). F_{ST} measures departures from random mating among populations and the statistic is based on allele frequencies. Secondly, the statistic, R_{ST}, devised by Slatkin (1995) was estimated whereby R_{ST} is based on the variance of allele size, and takes into account the mutation rates and specific mutation models at microsatellite loci. Finally, population subdivision was estimated by calculating G_{ST}, the coefficient of gene differentiation (Nei, 1973, 1987), also a frequency-based measure. $G_{ST} = D_{ST}/H_T$ where D_{ST} is the average gene diversity between samples and H_T is the gene diversity in the total population (Nei, 1973). These analyses were done by using the FSTAT (Goudet, 1995) and GENEPOP (Raymond and Rousset, 1995) programs.

To quantify the degree of differentiation among temporal samples relative to that among spatially different samples, a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed using ARLEQUIN ver 2.000 software (Schneider *et al.*, 2000). The first level of hierarchy consisted of samples from the Nguruman area and from the Lambwe Valley. The second level consisted of temporal or seasonal samples from each of these areas.

RESULTS

Allele frequencies, Hardy-Weinberg and Linkage disequilibrium

There was little variation in frequencies of the most common alleles at six loci in *G. pallidipes* collected during dry and wet seasons (Figure 1) at Nguruman. Allele frequencies were homogenous in the different seasons at Nguruman (Table I). Significant deviation (at P=0.05) of allele frequencies from Hardy-Weinberg equilibrium was observed in 13 of 54 tests (24%) for the dry season, and 13 of 78 in the wet season (17%).

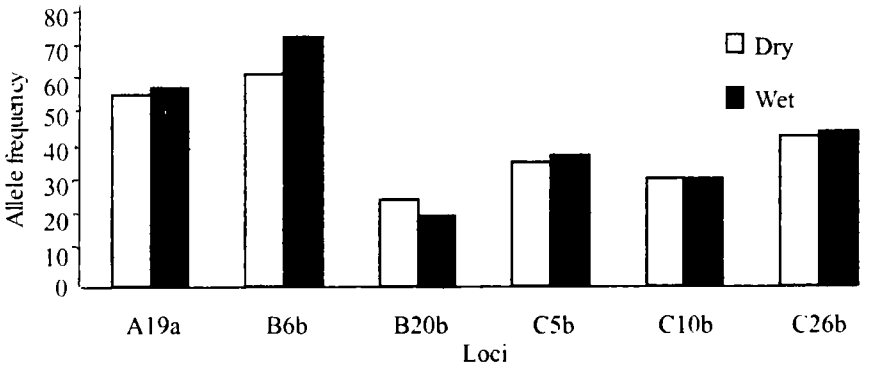

Figure 1. Frequency of most common allele at six loci for pooled G. pallidipes dry and wet season samples from the Nguruman, Kenya

TABLE I - ONE-WAY ANALYSIS OF VARIANCE FOR G PALLIDIPES SAMPLES FROM THE NGURUMAN

Locus	F-value[a]	P-value	X^2 b	P-value
Gp119a	0.25	0.623	0.04	0 849
GpA23b	0.57	0.465	0.49	0.483
GpB6b	0.11	0.746	0.63	0.429
GpB20b	0.01	0.912	0.89	0.346
GpC5b	1.60	0.217	1.35	0.245
GpC10b	2.33	0.133	0.34	0.561
GpC26b	1 68	0.202	1.13	0.289
GpCAG133	0.54	0 471	0.41	0.521

[a]Variance ratio

[b]Kruskal-Wallis test for homogeneity of allele frequencies among seasons, df =1

TABLE II - SINGLE LOCUS MICROSATELLITE DIVERSITIES OF *G. PALLIDIPES* IN NGURUMAN AND LAMBWE IN DRY AND WET SEASONS

Nguruman	Locus	*nall*	H_O	H_S	D_{ST}
Dry season	*GpA19a*	4	0.583	0.611	0.003
	GpB6b	7	0.679	0.524	0.032
	GpB20b	14	0.638	0.724	0.106
	GpC5b	10	0.450	0.676	0.018
	GpC10b	20	0.863	0.783	0.021
	GpC26b	11	0.724	0.700	0.018
	Mean± SE	11 ± 2.3	0.656 ± 0.057	0.670 ± 0.037	0.033 ± 0.015
Wet season	*GpA19a*	8	0.562	0.592	0.006
	GpB6b	8	0.501	0.437	0.009
	GpB20b	14	0.745	0.724	0.108
	GpC5b	5	0.495	0.653	0.006
	GpC10b	18	0.952	0.803	0.016
	GpC26b	12	0.720	0.720	0.005
	Mean± SE	10.8 ± 1.9	0.663 ± 0.073	0.655 ± 0.052	0.025 ± 0.017
Lambwe					
Dry season	*GpA19a*	6	0.572	0.585	0.041
	GpB6b	10	0.630	0.713	0.022
	GpC5b	12	0.437	0.680	0.006
	GpC26b	10	0.689	0.644	0.003
	Mean ± SE	9.5 ± 1.3	0.582 ± 0.054	0.655±0.027	0.018±0.009
Wet season	*GpA19a*	5	0.620	0.583	0.001
	GpB6b	7	0.483	0.609	0.002
	GpC5b	10	0.430	0.668	0.007
	GpC26b	9	0.740	0.683	0.006
	Mean± SE	7.8±1.1	0.568 ± 0.070	0.636 ± 0.024	0.004 ± 0.001

nall= number of alleles per locus, H_O=observed heterozygosity, H_S = expected heterozygosity D_{ST} = diversity between samples

All deviations in the dry and wet seasons corresponded to heterozygote deficiencies leading to positive F_{IS} values. F_{IS} measures departures from random mating within populations but here was caused by mutations in primer annealing sites. Only two of the 135 (1.5%) linkage disequilibrium tests were showed effect in the dry season. However, 10 out of 180 (5.6%) of the tests showed effect in the wet season. Linkage disequilibrium was not detected in any pair of loci, hence, all loci were considered statistically independent.

Microsatellite diversity

The mean number of alleles per locus of samples from Nguruman was 11 ± 2.3 in dry versus 10.8 ±1.9 in wet season (Table II). In Lambwe these were 9.5 ± 1.3 in dry versus 10.8 ± 1.9 in wet season. These values were not statistically different (P > 0.05). Total diversities, H_t in the dry and wet seasons were 0.703 and 0.680, respectively. Expected heterozygosities within samples, H_s did not differ in Nguruman (H_s = 0.670 ± 0.037 dry versus 0.655 ± 0.052 wet, t = 0.28, df = 10, P > 0.05) as well as in Lambwe (H_s = 0.655 ± 0.027 dry versus 0.636 ± 0.024 wet, t = 0.55, df = 6, P > 0.05). Diversity between dry season samples, D_{ST}, averaged 0.033 ± 0.015 in Nguruman (range: 0.003 – 0.106) and 0.018 ± 0.009 in Lambwe (Table II). Overall, diversities between dry-season samples were higher than those between wet-season samples though there was no statistical difference (Table II).

Genetic differentiation

Moderate differentiation was observed among Nguruman samples in both dry and wet seasons (F_{ST} = 0.051, G_{ST} = 0.047 in dry season) and (F_{ST} = 0.041, G_{ST} = 0.037 in the wet season) (Table III). Differentiation among Lambwe samples was slightly less than among Nguruman samples (F_{ST} = 0.028, G_{ST} = 0.026 in dry season; F_{ST} = 0.007, G_{ST} = 0.006 in wet season). However, dry season differentiation in Lambwe was four-fold higher than wet season differentiation. The magnitude of F_{ST} and G_{ST} differed among loci. Both F_{ST} and G_{ST} revealed higher levels of differentiation in the dry season than in the wet season. Generally, single locus and overall R_{ST} values were much lower than the corresponding F_{ST} and G_{ST} (Table III). These differences may be attributed to underlying assumptions of the various indices.

When dry season samples were pooled and compared with pooled wet season samples, F_{ST} was 0.008 and G_{ST} was 0.004 and R_{ST} was 0.0001. The foregoing values were not different from null expectations of zero (P >0.05). F_{IS} for pooled samples were 0.028 and 0.058 for dry and wet seasons, respectively. The increase in F_{IS} after pooling samples may indicate that demes of differing allelic frequencies were sampled, an example of the Wahlund effect.

Differentiation among samples was also assessed at different collection dates regardless of season (Table IV). Differentiation was low for the 1st collection, increased during the 2nd and 3rd collections, and dropped in the 4th and 5th collections. Overall differentiation among pooled samples for all collection dates was 0.025 ± 0.015.

To determine the magnitude of differentiation among temporal samples, we pooled samples to generate populations by years (date of collection). Pairwise F_{ST}s between temporal populations ranged from 0.001 between 2nd and 3rd collections to 0.039 between 3rd and 5th collections (Table V).

TABLE III - GENETIC DIFFERENTIATION OF NGURUMAN AND LAMBWE DRY AND WET SEASON SAMPLES OF *G PALLIDIPES*

Nguruman	Locus	F_{ST}	R_{ST}	G_{ST}
Dry season	*GpA19a*	0.006	0.010	0.004
	GpB6b	0.076	0.044	0.058
	GpB20b	0.121	0.043	0.128
	GpC5b	0.050	-0.001	0.027
	GpC10b	0.020	-0.006	0.026
	GpC26b	0.027	0.005	0.026
	All	0.051	0.006	0.047
Wet	*GpA19a*	0.006	0.000	0.010
	GpB6b	0.027	0.013	0.020
	GpB20b	0.137	0.028	0.130
	GpC5b	0.012	0.008	0.010
	GpC10b	0.021	0.013	0.019
	GpC26b	0.007	-0.001	0.007
	All	0.041	0.009	0.037
Lambwe				
Dry season	*GpA19a*	0.065	0.011	0.065
	GpB6b	0.036	0.007	0.030
	GpC5b	0.009	0.004	0.008
	GpC26b	0.005	0.001	0.005
	All	0.028	0.006	0.026
Wet season	*GpA19a*	0.002	0.002	0.002
	GpB6b	0.005	-0.007	0.003
	GpC5b	0.013	-0.005	0.010
	GpC26b	0.009	-0.004	0.008
	All	0.007	-0.003	0.006

TABLE IV - GENETIC DIVERSITY AND DIFFERENTIATION OF *G. PALLIDIPES* SAMPLES COLLECTED AT DIFFERENT DATES FROM NGURUMAN AND LAMBWE VALLEY

Date of Collection	*n*[*N*]	*MNA*	$H_O[H_E]$	$F_{IS}[F_{ST}]$	*P*
[1st]	2[88]	8.7	0.625[0.648]	0.035[0.014]	0.0006
[2nd]	4[80]	8.5	0.624[0.660]	0.056[0.050]	0.0001
[3rd]	4[87]	7.5	0.605[0.653]	-0.060[0.056]	0.8344
[4th]	3[96]	8.0	0.710[0.698]	-0.035[0.010]	0.9810
[5th]	4[161]	7.7	0.718[0.671]	-0.073[0.016]	0.7106

n = number of samples, *N* = number of individual tsetse, *MNA* = mean number of alleles per locus, H_O = observed heterozygosity, H_E = unbiased expected heterozygosity (Nei, 1987), *P* = unbiased estimate of Hardy-Weinberg exact *P*-value by the Markov Chain method under the alternative hypothesis of heterozygote deficit.

TABLE V - PAIRWISE F_{ST} ESTIMATES BETWEEN POOLED TEMPORAL SAMPLES FROM THE NGURUMAN

	1st	2nd	3rd	4th	5th
1st	—				
2nd	0.019	—			
3rd	0.032	0.001	—		
4th	0.025	0.031	0.033	—	
5th	0.019	0.038	0.039	0.005	—

TABLE VI - AMOVA FOR SEASONAL GENETIC VARIATION OF NGURUMAN SAMPLES (WEIR AND COCKERHAM 1984; EXCOFFIER *ET AL* 1992, WEIR 1996)

Source	d.f.	Variance	% variation	*F*	*P*[2]
Among seasons[1]	1	0.00681	0.33	$F_{CT} = 0.003$	> 0.05
Among populations within seasons	19	0.09038	4.41	$F_{SC} = 0.044$	$< 10^{-5}$
Within populations	1039	1.95258	95.26	$F_{ST} = 0.047$	$< 10^{-5}$
Total	1059	2.04978			

[1] Seasons refer to dry and wet

[2] Probability of obtaining a greater variance and F by chance.

TABLE VII - AMOVA OF NGURUMAN *G. PALLIDIPES* SAMPLES COLLECTED ON DATES

%Source		d.f.	Variance	variation	*F*	*P*[1]
Among dates[2]		4	0.03220	1.58	$F_{CT} = 0.015$	< 0.050
Among populations[3] within dates	12	0.05412	2.66	$F_{SC} = 0.027$	$< 10^{-5}$	
Within populations	1007	1.94932	95.76	$F_{ST} = 0.042$	$< 10^{-5}$	
Total	1023	2.03564				

[1] Probability of obtaining greater variance and F by chance

[2] Dates are May 2000, Nov. 2001, May 2003, Jul. 2003 and Dec. 2003.

[3] Populations refer to sampling sites.

Less than 1% of the total variance was attributed to the effect of seasons (Table VI). Most of the genetic variance (95%) was explained by differences among individuals within samples. About 4% of the variance was attributed to differences among samples within seasons. The corresponding *F*-statistics indicate unlimited gene flow between seasons ($F_{CT} = 0.003$) and among populations within seasons ($F_{SC} = 0.044$)

When samples were grouped according to collection date regardless of season, only 1.6% of the variation was attributed to differences in allele frequencies among sampling dates (Table VII). 2.7% of the variance was due to differences in frequencies among populations within dates. Most of the variance (~96%) lay within populations. F_{ST} estimated by the method of Weir and Cockerham (1984) was 0.042 whereas F_{SC} was 0.027. F_{SC} estimates the correlation of alleles in flies sampled on different dates and its low value indicates temporal stability in breeding structure.

DISCUSSION

Allele frequencies, Hardy-Weinberg and linkage disequilibrium

Frequencies of the most common alleles showed minimal variation between dry and wet seasons demonstrating that gene frequencies were homogenous between seasons. This temporal stability of allele frequencies indicates that seasonal genetic variation in *G pallidipes* was and no more than may be expected by genetic drift alone.

There was, however, indication of dSeveral factors could be responsible for the observed disequilibrium. First, *G. pallidipes* populations may exhibit population subdivision. Second, genetic drift could be acting on small resident populations hence causing disequilibria.

However, there is no historical evidence of a bottleneck in *G. pallidipes* populations in East Africa. Such evidence exists for South African *G. pallidipes* (Krafsur, 2002). Third, the observed pattern could be attributed to selection.

But microsatellites are generally thought to be selectively neutral (Schlötterer, 2000). However, selection would act if the loci in question are hitch-hicking to a gene or a functional region under selection (Slatkin, 1995). Fourth, disequlibrium may occur if the loci are physically linked. But this possibility is ruled out because linkage disequilibrium tests showed that the loci were independent. However, ultimate proof for non linkage can only come from mapping studies which was beyond the scope of this work. Finally, the observed disequlibrium could be as a result of null alleles.

Genetic differentiation

Genetic variability of *G. pallidipes* was assessed from temporal samples. Our data show that genetic differentiation fluctuates over time and is relatively higher in the dry season (F_{ST} = 0.051) than during the wet season (F_{ST} = 0.041). Although these values are not significantly different, the higher differentiation in the dry season can be attributed to drift. Pockets of *G. pallidipes* populations most likely survive in thickets where they can obtain blood meal from mammalian hosts. In the wet season, tsetse movement increases due to rise in humidity and fall in temperature (Brightwell *et al.*, 1992, 1997) leading to free exchange of genes. Williams *et al.* (1992) demonstrated that the mobility of *G. pallidipes* is greater during the rainy season than when it is dry. However, these results should be interpreted with caution because tsetse flies live long and generations overlap. Hence, dry season individuals are likely to be sampled in the wet season. It is possible that fairly isolated dry season demes admix in the wet season leading to a deficit in heterozygosity (Wahlund effect). Odulaja *et al.* (2001) observed that *G. pallidipes* catch distribution was more aggregated during the dry and long rainy seasons than in the short rainy season. The short rains experienced in November lead to a rise in humidity and a fall in temperature. These changes cause a more random distribution of tsetse and an increased dispersal capability (Odulaja *et al.*, 2001). It would therefore be expected that differentiation would be low during the short rains due to increased gene flow. Interestingly, F_{ST} value of 0.05 indicates reasonably high differentiation considering the small spatial scale covered. This result could be attributed to the hypothesis that dispersing insect populations sometimes move as groups and not as independent individuals (Stinner *et al.*, 1983, Odulaja, 2001). A more plausible cause of the high differentiation, however, could be genetic drift caused by small effective population numbers.

Comparison of different statistics to estimate population subdivision

Values of F_{ST} and G_{ST} were almost similar in magnitude because they are frequency-based measures. However, R_{ST} values for both dry and wet seasons were smaller than their F_{ST} counterparts. This was surprising especially given the assertion that under stepwise mutation model (SMM) or two-phase model (TPM), F_{ST} underestimates the degree of genetic differentiation among populations (Slatkin, 1995; Goodman, 1997). Thus smaller value of F_{ST} would be expected compared with that of R_{ST}. However, R_{ST} is biased by high mutation rates that lead to homoplasy. Therefore similar gel phenotypes are not identical by descent. Lower sensitivity of R_{ST} in comparison with F_{ST} was previously reported in humans (Perez-Lezaun *et al.*, 1997), bears (Peatkau *et al.*, 1997), and in *Anopheles gambiae* (Lehmann *et al.*, 1998). In a separate study, Ouma *et al.* (2005) observed higher differentiation by R_{ST} (0.229) than by F_{ST} (0.172) among 21 *G. pallidipes* populations obtained from East and southern Africa. It should, however, be noted that R_{ST} has high variance particularly when dealing with few loci (Gaggiotti *et al.*, 1999).

CONCLUSION AND RECOMMENDATIONS

The allele frequencies were homogenous across the period of study when the data was collected in 2001 and 2003. However, frequencies of the most common alleles varied slightly between seasons. Genetic diversities between dry season populations were higher than those between wet season populations. *G. pallidipes* populations were more genetically differentiated in the dry than in the wet season. Analyses of temporal samples regardless of season showed little temporal fluctuation in genetic diversities. It is concluded that microsatellite diversities are stable over seasons and time, and indicate that *G. pallidipes* populations do not undergo seasonal bottlenecks small enough to reduce allelic diversities detectably. Indeed, such temporal variation as detected is the consequence of genetic drift, which is inversely proportional to the numbers of breeding individuals.

The findings of this study are important for planning area-wide tsetse control strategies. Given the relatively high genetic differentiation of *G. pallidipes* populations in the dry season, targeting such isolated pockets in suppression efforts would

be more effective. The higher propensity of wet season samples to disperse would make them more difficult to target for elimination. On the other hand, it should be noted that lower tsetse densities are harder to kill and harder to detect relative to growing, thriving populations. And generation time in tsetse is sufficiently long such that control efforts should be maintained for years not weeks or months.

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