Genetic diversity of *Glossina pallidipes* in protected wildlife areas of Kenya

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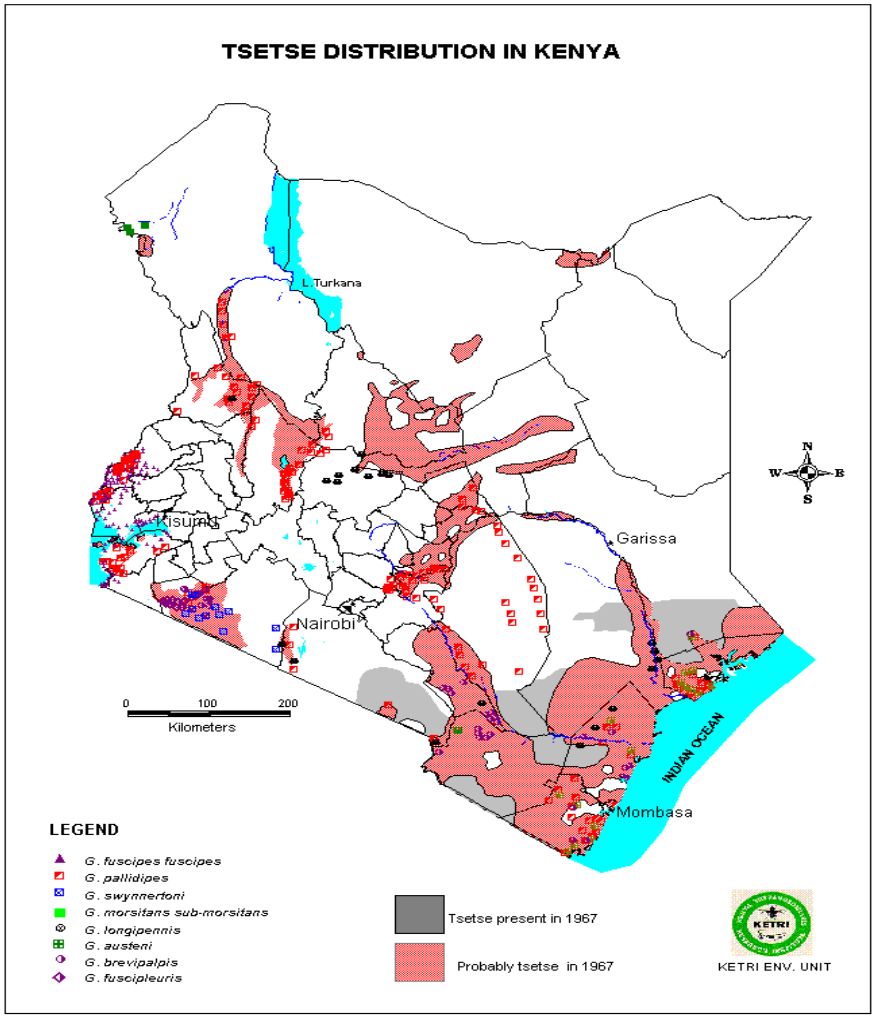
Introduction

- ~40% of Kenya is tsetse infested with 8 tsetse species
- Gpd is the most widely distributed species & is a transmitter of HAT and AAT
- Protected wildlife areas =National Parks & National Game Reserves (suitable tsetse habitats + blood meal source) hence a source of tsetse –re-infestation to farmlands
- PATTEC (K) initiative - area-wide eradication of Gpd in protected wildlife areas
- Tsetse eradication techniques include: traps, targets, live-baits, SAT & SIT and/or integrated approach
- Eradication programs- populations tend to recover- either flies survive the initial interventions or as a result of re-invasion
- Genetic baseline data generated will aid in area-wide tsetse eradication campaigns

Tsetse distribution in Kenya in 1996



Objectives

Specific objective

❑To generate genetic baseline data that can aid in area -wide tsetse eradication campaigns

Specific objectives

1. Determination of genetic diversity of Gpd populations
2. Estimation of genetic differentiation of Gpd populations

Methodology

Tsetse sampling

- Tsetse sampling- odour baited using biconical & NGU traps
- Trap sites geo-referenced – GPS (Garmin 12 XL)
- The trap coordinates were downloaded using Map source program and ArcMap version 10 Software used to produce distribution maps

Microsatellite loci

- 102 individuals were used for the genetic analyses at microsatellite loci: (28 in SHNR, 25 in MNP, 27 in MMNR, 5 in Narok and 17 in CHNP)
- Seven microsatellite loci were analyzed: (GpA19a, GmmC17, GpCAG133, GpB20b, GpC26b, GpB115 and GmmK06)
- Using genomic DNA as template, seven microsatellite loci were amplified via PCR

DNA extraction & amplification

- Genomic DNA was isolated from ethanol preserved Gpd samples using a GeneJet Genomic DNA purification kit
- PCR amplification was performed in a AB Applied Biosystems GeneAMP thermocycler as 13ul reactions containing 5x Phusion® HF buffer containing 7.5mM $MgCl_2$, 0.8µM each forward primer (labeled with FAM or VIC or PET or NED), and reverse primer, 0.2mM dNTPs, 0.1U Phusion® DNA Polymerase enzyme and 2µl of template DNA
- Primer sequences and PCR conditions were as described earlier (Baker and Krafsur, 2001; Ouma *et al*., 2003, 2006)
- The amplification profile consisted of initial denaturation at 98C for 5mins followed by 10cycles of 30s at 98C, 25s at 60C and 30s at 72C the 2nd step conditions were as follows 40 cycles of 98C for 30s, 50C for 25 s, 72C for 30sec and final extension at 72C for 20mins.

Sequencing & genotyping

- The PCR products were co-loaded as sets of two, set 1 consisted of Loci 1, 2, 3 and set 2 loci 4, 5, 6 and 7
- The pooled products were added to 9ul cocktail mix (1000µl Hi-Di and 12µl of Liz 500)
- This was denatured at 95C and quickly chilled on ice for 3mins
- Analysis of fragment size was performed using the Genetic Analyzer 3730
- Genotype of individual flies was then determined using Genemapper version 3.7.

Microsatellite data Analysis

- Estimates of mean no. alleles per locus, observed and expected heterozygosities were calculated using FSTAT (Goudet, 2001)
- Hardy-Weinberg Equilibrium and genotypic linkage disequilibrium at microsatellite loci were performed using GENEPOP version 3.4 (Raymond and Rousset, 1995)
- Genotyping errors and null allele frequencies were determined using Micro-Checker software
- Genetic differentiation within and among populations was estimated by calculating F-statistics and FSTAT
- Measures of departures from random mating (FIS) and among (FST) were estimated

Genetic diversity

Population Statistics								
Population	Sample size	Loci typed	Unbiased Hz	Unbiased Hz SD	Obs Hz	Obs Hz SD	No Alleles	No Alleles SD
Meru	25	7	0.6725	0.1066	0.4717	0.0420	5.43	2.23
Mara	27	7	0.6331	0.0675	0.5099	0.0369	6.29	2.69
Narok	5	7	0.5822	0.1136	0.5714	0.0849	3.71	1.80
Chyulu	17	7	0.5067	0.1197	0.3813	0.0455	4.14	2.54
Shimba	28	7	0.5868	0.0610	0.4121	0.0370	5.14	1.95
			0.5963				**4.94**	**2.24**

Genetic diversity

- All the five fly populations seem have high diversity with heterozygosity (unbiased **Hz >50%)**
- Mean number of alleles per locus = **4.94**
- Mean average unbiased heterozygosity = **0.596 (~60%)**
- Meru National Park & Masai Mara National Reserve have the most diverse populations
- It seems there is a high genetic diversity of Gpd populations in the protected wildlife area

Genetic differentiation

Locus	Fwc(is)	Fwc(st)	Fwc(it)
GpA19a	0.0989	0.0989	0.1880
GmmC17	0.5360	0.1523	0.6067
GpCAG133	0.2058	0.1879	0.3551
GpB20b	0.4920	0.0163	0.5003
GpC26b	0.0946	0.0408	0.1316
GpB115	0.3060	0.2312	0.4665
GmmK06	0.0317	0.1523	0.1792
All:	**0.2635**	**0.1169**	**0.3496**

Genetic differentiation

- Overall, high genetic differentiation with average Fst across 7 loci and five populations is 0.1169 **(~0.117)**
- High Fis **(~0.264)** indicates that protected wildlife areas harbor heterogenous populations
- It seems there is limited gene flow in the protected wildlife areas hence populations are isolated
- Such isolated populations are easy to target individually for eradication/elimination

Conclusion

Gpd populations in protected wildlife areas are genetically isolated hence can be targeted for elimination


Thank YOU
FOR LISTENING