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 2\(^{nd}\) 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 9µl cocktail mix (1000µl Hi-Di and 12µl of Liz 500)
- This was denatured at 95°C 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

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Loci typed</th>
<th>Unbiased Hz</th>
<th>Unbiased Hz SD</th>
<th>Obs Hz</th>
<th>Obs Hz SD</th>
<th>No Alleles</th>
<th>No Alleles SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meru</td>
<td>25</td>
<td>7</td>
<td>0.6725</td>
<td>0.1066</td>
<td>0.4717</td>
<td>0.0420</td>
<td>5.43</td>
<td>2.23</td>
</tr>
<tr>
<td>Mara</td>
<td>27</td>
<td>7</td>
<td>0.6331</td>
<td>0.0675</td>
<td>0.5099</td>
<td>0.0369</td>
<td>6.29</td>
<td>2.69</td>
</tr>
<tr>
<td>Narok</td>
<td>5</td>
<td>7</td>
<td>0.5822</td>
<td>0.1136</td>
<td>0.5714</td>
<td>0.0849</td>
<td>3.71</td>
<td>1.80</td>
</tr>
<tr>
<td>Chyulu</td>
<td>17</td>
<td>7</td>
<td>0.5067</td>
<td>0.1197</td>
<td>0.3813</td>
<td>0.0455</td>
<td>4.14</td>
<td>2.54</td>
</tr>
<tr>
<td>Shimba</td>
<td>28</td>
<td>7</td>
<td>0.5868</td>
<td>0.0610</td>
<td>0.4121</td>
<td>0.0370</td>
<td>5.14</td>
<td>1.95</td>
</tr>
</tbody>
</table>

Mean: **0.5963**

SD: **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 $= 0.596$ ($\sim 60\%$)
• 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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Fwc(is)</th>
<th>Fwc(st)</th>
<th>Fwc(it)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GpA19a</td>
<td>0.0989</td>
<td>0.0989</td>
<td>0.1880</td>
</tr>
<tr>
<td>GmmC17</td>
<td>0.5360</td>
<td>0.1523</td>
<td>0.6067</td>
</tr>
<tr>
<td>GpCAG133</td>
<td>0.2058</td>
<td>0.1879</td>
<td>0.3551</td>
</tr>
<tr>
<td>GpB20b</td>
<td>0.4920</td>
<td>0.0163</td>
<td>0.5003</td>
</tr>
<tr>
<td>GpC26b</td>
<td>0.0946</td>
<td>0.0408</td>
<td>0.1316</td>
</tr>
<tr>
<td>GpB115</td>
<td>0.3060</td>
<td>0.2312</td>
<td>0.4665</td>
</tr>
<tr>
<td>GmmK06</td>
<td>0.0317</td>
<td>0.1523</td>
<td>0.1792</td>
</tr>
<tr>
<td><strong>All:</strong></td>
<td><strong>0.2635</strong></td>
<td><strong>0.1169</strong></td>
<td><strong>0.3496</strong></td>
</tr>
</tbody>
</table>
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.