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Breeding structure of *Glossina pallidipes* (Diptera: Glossinidae) populations in East and southern Africa

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Abstract

Gene diversity and gene flow in *Glossina pallidipes* Austen were studied by using allozyme electrophoresis on samples from southern and East Africa. Recorded were 30 alleles segregating at eight loci. Gene diversity was 0.212 ± 0.085 S.E. in four southern African populations and 0.163 ± 0.076 in seven Kenyan populations. All loci were in Hardy-Weinberg equilibrium. *Pgm* and *6pgd* were sex linked. Spatial components of gene diversity were measured by using *F* statistics. Mating was random within each population. The 'fixation index' F_{ST} was 0.133 ± 0.062 among southern African populations. Among the Kenyan populations, F_{ST} was 0.159 ± 0.069. F_{ST} was 0.238 ± 0.051 among all populations. Analysis of variance of gene frequencies showed that 65.8% of the genetic variance lay within populations and 34.2% of the genetic variance lay between Kenyan and southern African populations. These data suggest a strong measure of genetic drift among tsetse populations even in a region where it is thought they are continuously distributed. The causes of this drift require investigation.

Introduction

Glossina pallidipes Austen (Diptera: Glossinidae) is a member of the *G. morsitans* Westwood species group and inhabits forests, woodland savannas, and semiarid thickets. The geographic distribution of *G. pallidipes* extends from Malawi, Mozambique, Zimbabwe, and Zambia north to Sudan, Ethiopia, and Somalia; Tanzania, Congo, Kenya, and Uganda also harbour populations (Jordan, 1993). In East Africa, some populations at least are strongly disjunct (fig. 1).

Behaviour and host preferences in *G. pallidipes* can vary geographically (Torr *et al.*, 1988; Colvin & Gibson, 1992), but the genetic basis of such variation, if any, is unknown. Polymorphisms in supernumerary 'B' chromosomes were detected in *G. pallidipes* (Maudlin, 1970). A paracentric inversion was found in a laboratory culture of *G. pallidipes* and the somatic, meiotic, and polytene chromosome complement has been described (Southern & Pell, 1981).

Glossina morsitans morsitans (Machado) showed chromosomal polymorphisms that correlated with strain variations in laboratory performance (Jordan *et al.*, 1977) but unreplicated correlations in three samples do not necessarily have a genetic basis. The existence of genetically distinct conspecific tsetse populations, if real, raises the question of gene flow within and among populations. If rates of dispersal and immigration are high, then local genetic differentiation is unlikely unless selection is strong. The reproductive potential of tsetse is such that strong selective regimes are likely to reduce substantially population densities because tsetse do not have much excess reproductive capacity. It is generally conceded that tsetse are highly vagile.

Estimates of how genetic diversity is partitioned within and among demes ('breeding structure') may in principle suggest how species track continuously varying environments and adapt to local conditions in the face of gene flow among the diverse populations (Wright, 1978; Nei, 1987; Hartl & Clark, 1989). Research has demonstrated the presence of endosymbionts (O'Neill *et al.*, 1993) and transposable elements (Blanchetot & Gooding, 1995) that could, in principle, act as genetic isolating mechanisms, because hybrid matings may be sterile or semisterile. Tsetse have low reproductive rates, long generation times, and occupy habitats that are isolated by distance from other,

similar habitats, show polymorphisms in behaviour (Ford *et al.*, 1972), and may carry heritable factors that confer a measure of reproductive isolation. It is therefore important to know just how 'local' are tsetse populations, and how much gene flow exists between them.

Earlier work on *G. pallidipes* includes a survey of allozymes among eight Kenya populations (Van Etten, 1982) in which 11 presumptive loci were described, three of which were polymorphic. No inferences were drawn about gene flow. Agatsuma & Otieno (1988) examined 11 enzymes in two Kenyan *G. pallidipes* populations. Phosphoglucomutase and phosphoglucoisomerase were highly polymorphic but the data were not tested for gene flow. Gene diversity at 12 putative loci was examined in two disjunct *G. pallidipes* populations in Kenya and significant differences in gene frequencies and gene diversities were recorded (Nesbitt *et al.*, 1990).

A project on regional variation in behaviour of tsetse to attractant chemicals and trapping devices (Griffiths & Brady, 1994) provided an opportunity to collect flies from diverse locations for genetic analysis. We now present the genetic analyses.

Methods

Sampling

Glossina pallidipes were collected in Zimbabwe and Mozambique in August and September, 1994. Collections were made in Kenya in August, 1995 (fig. 1).

Zimbabwe tsetse were collected at Rekomitjie, under the southern escarpment of the Zambezi valley; Makuti, 20 km south of Rekomitjie, and 300 m higher in elevation; and Mana Pools, 40 km north of Rekomitjie. The foregoing three collections were from a region with abundant game, and without a history of human settlement or agriculture. The Mozambique sample was collected in Changara, 20 km east of Nyampanda, 300 km downstream from Rekomitjie. There was little or no human activity in Changara.

Sampling in Kenya included three districts east of the Rift Valley and two districts west of it. The mountainous terrain of much of Kenya has created natural discontinuities in tsetse distribution to which must be added those discontinuities in suitable tsetse habitat caused by human settlements and agriculture.

A collection of flies from a particular sampling site and day were killed, identified, sexed, placed in labelled cryostat vials, and dropped into liquid nitrogen. Later, flies were shipped in liquid nitrogen to Imperial College at Sunninghill and thence to Ames, Iowa for genetic analysis.

Electrophoresis

Polyacrylamide gel electrophoresis was used to separate enzymatic proteins from homogenized tsetse. A single tsetse (less its head) was homogenized in 300 µl of grinding buffer containing sucrose, bromophenol blue, dithiothreotol, pH 8.6 tris-citrate buffer, and basic fuschsin (Black & Krafsur 1985a). Gels consist of 6.18% acrylamide plus 0.325% bis- acrylamide, 0.05% ammonium persulfate, and 0.15% TEMED in gel buffer.

The buffer systems for anodally migrating enzymes included modified Ornstein-Davis (Black & Krafsur 1985a) without the stacking gel, tris-borate EDTA pH 8.9 (Munstermann, 1979), and NAM-citrate pH 6.5 (Clayton & Tretiak, 1972). The TBE electrode and gel buffer consist of 81 mM Tris, 20 mM borate, and 1.5 mM disodium EDTA. The NAM-citrate gel buffer is 2.7 mM citrate, and the electrode buffer is 5.35 mM citrate; both are adjusted to pH 6.5 with N-(3-aminopropyl)-morpholine.

Hoefer SE600 gel boxes were used for vertical slab electrophoresis. 2.5 µl of sample homogenate was applied to each of 28 sample wells per gel. NAM gels were run at a constant 30 mA per gel; TBE and OD gels were run at a constant 350 V (30 V per cm). Electrophoresis was continued until the bromphenol tracking dye had reached 80 mm from the origin.

Staining recipes for most allozymes are in Murphy *et al.* (1990) with numerous modifications over the years. For stains requiring G6pdh or other coupling enzymes, we routinely used agar overlays, with 5 units of G6pdh, 0.5 mg PMS, 2 mg NAD (or 2 mg NADP, as appropriate) and 1 mg MTT (15–20 ml total volume is sufficient to cover two gel slices). We also used agar overlays to stain for the peptidase. For other stains, we used 5 mg of NAD or NADP, 5 mg MTT, and 1 mg PMS per 100 ml of stain solution.

Interpretation of gels

Allozyme subunit structures were determined by the banding patterns of heterozygotes. Bands were measured from their points of origin and the values recorded. A permanent record was obtained by photographing gels with Kodak Gold 100 colour film.

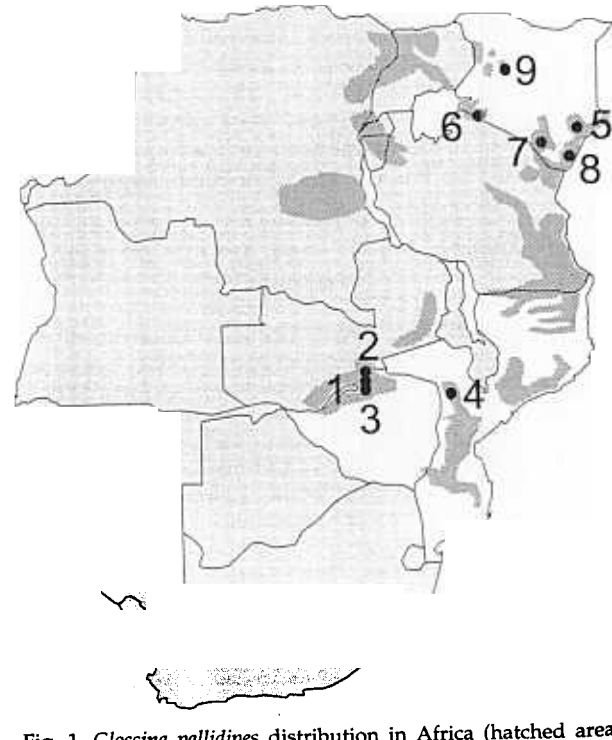

Fig. 1. *Glossina pallidipes* distribution in Africa (hatched areas, after Ford, 1971) and approximate sampling locations. 1, Rekomitjie; 2, Mana Pools; 3, Makuti; 4, Changara; 5, Galana (Alangoshira, Dakabuko); 6, Nguruman (Nguruman, Shompole); 7, Kibwezi; 8, Shimba Hills; 9, Marech Pass.

Genetic statistics

For the sex linked loci, 6-phosphoglucodehydrogenase (*6pgd*) and phosphoglucomutase (*Pgm*), genotypes from only females were submitted for analysis because the males were hemizygous. Nei's (1987) prescriptions were used to compute gene diversities and variances. Gene diversity at a locus is measured by the statistic $h_e = 1-p_i^2$, where p_i is each putative allele at the locus. Gene diversity for n loci is $H_E = [\Sigma(h_e)/n$, with variance $= \Sigma(h_e-H_E)^2/[n(n-1)]$. In simple terms, H_E and h_e are the expected heterozygosities, on a scale of 0 to 1.0, when mating is random and other Hardy-Weinberg assumptions apply. Chi-square tests of homogeneity of gene frequencies used the methods of Workman & Niswander (1970). Gene frequency data were analysed by using *Biosys-1* (Swofford & Selander, 1981) and *Genestats* (Black & Krafsur, 1985b). Use of Wright's *F*-statistics and their modifications is the most efficacious way to describe the breeding structure of subdivided populations (Slatkin, 1985, 1987; Nei, 1987). *F* estimates departures from random mating; the basic relationship is, $F = (h_e-h_0)/h_e$, which compares the observed heterozygosity at a selectively neutral locus h_0 to the expected heterozygosity h_e under Hardy-Weinberg criteria of an ideal closed population of infinite size, a negligible mutation rate, and discrete generations. Because natural populations are more or less discontinuously distributed in space as demes (Wright, 1969), individuals in populations ordinarily will not have an opportunity for truly random mating; they may be of different ages, or under different selective pressures, there will be dispersal, and so forth. Thus populations will be structured and the breeding composition will vary spatially and temporally.

Departures from random mating within subpopulations are indicated by F_{IS}; a deficiency of heterozygotes gives a positive estimate while an excess will give a negative F_{IS} estimate. Departures from random mating among subpopulations are estimated by F_{ST}. A significant F_{ST} can be interpreted as a measure of reproductive isolation and Wright termed it the 'fixation index', the amount of differentiation relative to the limiting amount under complete fixation. F_{IT} estimates departures from random mating in individuals relative to the whole population. The statistics are related as $F_{IT} = F_{ST} + F_{IS} - (F_{IS})(F_{ST})$.

F_{ST} measures the effects of random drift and differential selection among subpopulations and for selectively equivalent variation at each locus, it measures only drift. According to the island model of Wright (1969), the statistic can be used to estimate the average level of gene flow in terms of the equivalent mean number of migrants per subpopulation per generation. Assuming that subpopulations are at equilibrium with respect to drift and migration, the model is, $F_{ST} \approx 1/(1 + 4Nm)$. Although estimates from this model are based on an island model of population structure, they have been found to be reasonable approximations for the opposite extreme of stepping-stone arrays (Slatkin, 1987). F_{ST} approaches its equilibrium value rapidly (Crow & Aoki, 1984) unless pronounced founder effects caused the initial divergence among subpopulations.

The statistical significance of *F* statistics was evaluated by using chi-square. For loci with k alleles in s populations, $\chi^2 = 2NF_{ST}(k-1)$ with $(s-1)(k-1)$ degrees of freedom tests the hypothesis that $F_{ST} = 0$ (Workman & Niswander, 1970).

Results

Gene diversity

A survey of 40 presumptive genetic loci disclosed that only 11 were polymorphic (27.5%). A number of polymorphic loci presented banding patterns that were clearly interpretable by Mendelian criteria, but unaccountably could be not resolved in one or more samples well enough to be scored. Such loci included hexokinase (EC 2.7.1.1), trehalase (EC 3.2.1.28), and tyrosine aminotransferase (EC 2.6.1.5). 'Rare' alleles, defined as being present in mean frequencies <0.05, were detected at aconotate hydratase (*Aco*, EC 4.2.1.3), formaldehyde dehydrogenase (*Form*, EC 1.2.1.1), *Pgm* (EC 5.4.2.2), and triose-phosphate isomerase (*Tpi*, EC 5.3.1.1). Only loci that were in Hardy-Weinberg proportions in each sample were analysed further. A sum of 30 alleles segregating at eight loci were scored (table 1).

Pgm and *6pgd* (EC 1.1.1.44) were sex linked. Males showed only a single band whereas many females showed two (*Pgm*) or three (*6pgd*) bands in heterozygotes. Allele frequencies from females only were used to examine gene flow at the sex linked loci. The mean expected and observed heterozygosities at each sampling location are set forth in table 2. In Kenya, $H_E = 0.163 \pm 0.075$, and in southern Africa, $H_E = 0.212 \pm 0.085$. The two estimates did not differ significantly ($t = 0.43$, $P = 0.67$). *6pgd* was polymorphic in three of the southern African populations but monomorphic in the seven Kenyan samples. Kenyan samples showed less diversity than the southern populations particularly at *Form* and *Had*.

Gene flow within and among populations

Southern Africa G. pallidipes

Allele frequencies differed greatly among the four samples as shown by contingency chi-square; only three of the eight loci were homogeneous among samples (populations), these being *Aco*, *Pgm*, and *Tpi*. Contingency tests among the Zimbabwe samples were also highly significant when summed over the eight loci (table 3).

Breeding structure of the southern African *G. pallidipes* populations is described by the *F* statistics (table 4). A slight, but nonsignificant, excess of heterozygotes was detected within populations (F_{IS}). Mating among populations, however, was not random, as indicated by a large F_{ST} estimate. The mean immigration rate per population estimated by F_{ST} is approximately equivalent to two reproducing flies per generation if populations were 'ideal'.

Analysis of variance of gene frequencies showed that 69.1% of the total variance was attributed to the Zimbabwe populations and 30.9% of the variance was attributed between Zimbabwe and Mozambique.

Kenya G. pallidipes

Two samples from each of two districts were compared by using contingency tests. Both the Galana and Nguruman samples were homogeneous. These data suggest that matings were random in districts and that populations have radii of at least 15–30 km. At a higher hierarchical level, samples from three districts east of the Rift Valley were compared with samples from two districts west of the Rift.

Table 1. Gene frequencies in southern African and Kenyan *Glossina pallidipes* populations.

	Southern Africa			Kenya						
Locus	Rekomitjie	Mana Pools	Makuto	Changara	Dakabuko	Alangoshira	Shimba	Kibwezi	Nguruman	Shompole	Marech
		56	24	48	31	56	21	28	56	56	28
		0.000	0.000	0.000	0.000	0.018	0.000	0.125	0.000	0.000	0.000
		0.000	0.000	0.021	0.032	0.018	0.000	0.089	0.000	0.000	0.054
		1.000	1.000	0.979	0.952	0.946	1.000	0.732	1.000	1.000	0.946
		0.000	0.000	0.000	0.016	0.018	0.000	0.054	0.000	0.000	0.000
		55	24	50	31	56	21	28	56	56	28
		0.000	0.021	0.000	0.016	0.018	0.000	0.036	0.000	0.000	0.000
		0.991	0.938	1.000	0.984	0.946	1.000	0.964	1.000	1.000	1.000
		0.009	0.042	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000
		56	22	50	30	56	20	28	56	56	28
		0.000	0.000	0.000	0.017	0.018	0.000	0.000	0.000	0.000	0.000
		0.107	0.136	0.130	0.133	0.045	0.050	0.018	0.000	0.000	0.000
		0.893	0.841	0.840	0.767	0.839	0.875	0.946	1.000	1.000	0.982
		0.000	0.023	0.030	0.033	0.027	0.000	0.036	0.000	0.000	0.018
		0.000	0.000	0.000	0.000	0.054	0.075	0.000	0.000	0.000	0.000
		0.000	0.000	0.000	0.050	0.018	0.000	0.000	0.000	0.000	0.000
		56	24	50	31	30	21	28	56	56	28
		0.027	0.125	0.030	0.000	0.033	0.071	0.000	0.000	0.000	0.000
		0.973	0.875	0.960	0.984	0.950	0.929	0.982	1.000	1.000	0.946
		0.000	0.000	0.010	0.016	0.017	0.000	0.018	0.000	0.000	0.054
		52	24	49	31	59	21	28	28	28	26
		0.000	0.000	0.143	0.032	0.025	0.024	0.036	0.018	0.089	0.000
		0.067	0.125	0.092	0.048	0.076	0.238	0.339	0.411	0.464	0.308
		0.058	0.125	0.214	0.790	0.763	0.619	0.446	0.571	0.446	0.327
		0.529	0.583	0.051	0.097	0.119	0.119	0.107	0.000	0.000	0.308
		0.077	0.125	0.480	0.016	0.017	0.000	0.071	0.000	0.000	0.058
		0.269	0.042	0.020	0.016	0.000	0.000	0.000	0.000	0.000	0.000
		56	22	50	24	25	11	14	27	28	14
		0.955	0.955	0.960	0.833	0.860	0.955	0.857	0.389	0.429	1.000
		0.045	0.045	0.040	0.167	0.120	0.045	0.143	0.611	0.571	0.000
		0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
		25	24	26	31	56	21	28	56	56	28
		0.340	0.000	0.288	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		0.660	1.000	0.712	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	10	56	24	50	31	56	21	28	56	56	28
	0.000	0.018	0.021	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
	1.000	0.964	0.917	1.000	1.000	0.982	1.000	0.982	1.000	1.000	1.000
	0.000	0.018	0.063	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000

An analysis of variance showed that essentially all the genetic variance was attributed to districts within regions (east and west). The *F* statistics show that, averaged over loci, mating was random within populations (table 5). Indeed, five loci showed a slight excess of heterozygotes. Mating was not random among the Kenyan populations ($F_{ST} = 0.156$), translating to an equivalent of 1.4 reproducing immigrants per population per generation.

Contrasts between southern Africa and Kenya

Hierarchical *F* statistics averaged over all populations (table 6) showed random mating within populations, but not among populations. The analysis of variance showed that 34.2% of the total was attributed to differences between the southern and northern populations (table 7). This difference translates to an $F_{ST} \approx 0.075$. When the data were pooled into two groups, Kenya and southern Africa, an F_{ST} of 0.107 was estimated by using Nei's (1977) formula; by Weir & Cockerham's (1984) method, $F_{ST} = 0.219 \pm 0.055$.

Discussion

Chromosome diversity, in the number of supernumerary 'B' chromosomes, and in a 'floating' paracentric inversion has been described qualitatively (Maudlin, 1970; Southern, 1981) but the numerical and geographical patterns of this diversity have not been elucidated. Diversity at electrophor-

Table 2. Observed and expected heterozygosities at eight loci in *Glossina pallidipes* populations.

Population	Mean sample size per locus[a]	Mean no. of alleles per locus[a]	Percentage of loci polymorphic	Mean heterozygosity	
				Observed[a]	Expected[a,b]
1. Rekomitjie	35.5 (5.7)	2.4 (0.4)	87.5	0.217 (0.062)	0.228 (0.068)
2. Mana Pools	51.5 (3.8)	2.4 (0.4)	87.5	0.208 (0.093)	0.190 (0.083)
3. Makuto	23.5 (0.3)	2.5 (0.5)	75.0	0.191 (0.075)	0.187 (0.071)
4. Changara	46.6 (3.0)	2.5 (0.6)	75.0	0.205 (0.094)	0.199 (0.088)
5. Dakabuko	30.0 (0.9)	2.8 (0.6)	75.0	0.152 (0.060)	0.151 (0.060)
6. Alangoshira	49.3 (4.8)	3.4 (0.6)	87.5	0.167 (0.052)	0.161 (0.049)
7. Shimba	19.6 (1.2)	1.9 (0.4)	50.0	0.126 (0.065)	0.127 (0.069)
8. Kibwezi	26.3 (1.8)	2.6 (0.5)	87.5	0.201 (0.080)	0.203 (0.086)
9. Nguruman	48.9 (4.7)	1.4 (0.3)	25.0	0.113 (0.075)	0.125 (0.082)
10. Shompole	49.0 (4.6)	1.4 (0.3)	25.0	0.121 (0.082)	0.136 (0.089)
11. Marech	26.0 (1.7)	1.8 (0.4)	50.0	0.127 (0.093)	0.120 (0.086)

[a]Standard errors are in parentheses.
[b]By Hardy-Weinberg criteria: unbiased estimate of Nei (1978)

etic loci can be ideal for studying patterns of gene flow provided that the loci examined are selectively neutral, unambiguously scorable, and sufficiently polymorphic. Nesbitt *et al*. (1990) examined two *G. pallidipes* populations in Kenya and found that gene frequencies at *esterase*, *Pgi* and *Pgm* differed significantly between populations.

No alleles were found that discriminated Kenyan from the southern African *G. pallidipes*. It was principally differences in gene frequencies that separated populations, and most genetic variation lay within populations.

We observed the average gene diversity to be greater in southern Africa than in Kenya, but not significantly so. Tsetse habitat in the Zambezi valley is uninterrupted, and it is thought that populations there are continuously distributed. Moreover, there is no history of large scale clearing of bush or reduction of game, so tsetse 'effective population sizes', in the genetic sense (Nei, 1987), have been large compared with those in East Africa. *Glossina pallidipes* populations in Kenya, on the other hand, were discontinuously distributed, being interrupted by large areas of cleared land and dense human populations. Added to this are the gaps provided by the Rift Valley, and the severe land profile in which there are many natural areas devoid of mammalian hosts and sufficient vegetation, where tsetse populations cannot be sustained. Distribution maps show that *G. pallidipes* populations are disjunct in much of Kenya but more continuous in southern Africa (Jordan, 1993). Thus most tsetse habitats in Kenya are smaller than in southern Africa with the consequence that tsetse populations are also smaller in magnitude.

The foregoing scenario, in which gene diversity is directly proportional to effective population size, is supported by the *F* statistics. Genetic drift (F_{ST}) was more pronounced in the East African tsetse, but it is important to note that subsampling each of two *G. pallidipes* populations showed no genetic differentiation within them. Thus we were able to show that populations have radii that are at least 15 to 30 km. Moreover, mating was random within

Table 3. Contingency chi-square analysis for homogeneity of allele frequencies among *Glossina pallidipes* populations, summed over eight loci.

Source	No. populations	Chi square	df	*P*
Kenya		458.582		0.00000
(Galana	2	19.98		0.459)
(Nguruman	2	3.89		0.274)
Southern Africa	4	294.998		0.00000
(Zimbabwe	3	84.642		0.00000)
Totals	11	1691.559		0.00000

Table 4. *F* statistics for southern Africa *Glossina pallidipes* populations.

Locus	F_{IS}	F_{ST}	F_{IT}
Aco	−0.0067	0.0005	−0.0061
Form	−0.0538	0.0432***	−0.0083
Had	0.0369	0.0081	0.0448
Pep	−0.0091	0.0296***	0.0208
6pgd	−0.0243	0.1058***	0.0841
Pgi	−0.0236	0.1931***	0.1740
Pgm	−0.0398	−0.0096	−0.0498
Tpi	−0.0351	0.0230**	−0.0114
Mean	−0.0145	0.1123	0.0994
Jackknife estimates:			
Mean	−0.0157	0.1331	0.1189
S.D.	0.0116	0.0620	0.0562

**$P \leq 0.005$.
***$P \leq 0.001$.

able 5. *F* statistics for Kenya *Glossina pallidipes* populations.

Locus	F_{IS}	F_{ST}	F_{IT}
Aco	–0.0393	0.0940***	0.0584
Form	–0.0261	0.0179	–0.0077
Had	–0.0062	0.0714***	0.0656
Pep	–0.0360	0.0242*	–0.0109
6pgd	0.000	0.000	0.000
Pgi	0.0101	0.1082***	0.1172
Pgm	0.0875	0.2875***	0.3499
Tpi	–0.0053	0.0034	–0.0109
Mean	0.0229	0.1560	0.1753
Jackknife estimates:			
Mean	0.0236	0.1590	0.1806
S.D.	0.0259	0.0695	0.0906

*$P \leq 0.02$.
***$P \leq 0.000$

populations as shown by average F_{IS} estimates that did not differ significantly from zero. F_{IS} would have been positive if sampling had included two or more demes that differed in their gene frequencies, the 'Wahlund effect' (Hartl & Clark, 1989).

Restriction of gene flow among populations in both southern and East Africa was rather greater than we had anticipated. Ecological studies had suggested that tsetse were highly mobile (Jordan, 1993). Indeed, the amount of genetic drift in *G. pallidipes* populations, as indicated by F_{ST}, is very great for conspecific populations and may be suggestive of a measure of postmating reproductive isolation, unless, of course, this species is much less vagile than supposed. Possible isolating mechanisms include sex chromosome aneuploidy (Maudlin, 1979), the presence of one or more active transposable elements, and *Wolbachia pipientis* (O'Neill *et al.* 1993).

The data suggest some gene flow between northern and southern Africa: the F_{ST} of 0.075–0.107 estimates an exchange rate equivalent to 2–3 reproducing flies per generation. Langley *et al.* (1984) hybridized *G. pallidipes* field collected flies from Zimbabwe and laboratory flies descended from material collected from Uganda. There were no incompatibilities but differences between strains were seen in supernumerary chromosomes and in heterochromatin amounts. Rather wider sampling of tsetse is necessary, however, to test adequately the hypothesis that there are no premating or postmating reproductive barriers. Our results clearly refute the null hypothesis that mating is random among *G. pallidipes* populations while confirming random matings within populations. These results may be of importance when area-wide control measures are applied because they may indicate that there exist premating barriers between released, sterile or genetically transformed tsetse and the target form.

Table 6. *F* statistics for all *Glossina pallidipes* populations.

Locus	F_{IS}	F_{ST}	F_{IT}
Aco	–0.0358	0.0861***	0.0534
Form	–0.0390	0.0263**	–0.0117
Had	0.0179	0.0496***	0.0666
Pep	–0.0165	0.0397***	0.0239
Pgi	–0.0064	0.2649***	0.2602
Pgm	0.0529	0.3182***	0.3543
6pdg	–0.0291	0.2733***	0.2521
Tpi	–0.0275	0.0200**	–0.0069
Mean	0.0014	0.2201	0.2211
Jackknife estimates:			
Mean	0.0001	0.2383	0.2383*
S.D.	0.0118	0.0506	0.0498

**$P \leq 0.001$.
***$P \leq 0.0001$

Table 7. Nested analysis of variance of gene frequencies in all populations.

Comparison	Variance component	
	0.19372	65.8
	0.10054	34.2
	0.29426	

*Regions refer to Kenya and southern Africa.

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