Published in final edited form as:
Med Vet Entomol. 2002 September ; 16(3): 292–300.

Microsatellite diversities and gene flow in the tsetse fly, *Glossina morsitans s.l.*

E. S. KRAFSUR and **M. A. ENDSLEY**
Department of Entomology, Iowa State University, Ames, U.S.A

Abstract

Tsetse flies occupy discontinuous habitats and gene flow among them needs to be investigated in anticipation of area-wide control programs. Genetic diversities were estimated at six microsatellite loci in seven *Glossina morsitans submorsitans* Newstead (Diptera: Glossinidae) populations and five microsatellite loci in six *G. m. morsitans* Westwood populations. Nei's unbiased diversities were 0.808 and 76 alleles in *G. m. submorsitans* and 0.727 and 55 alleles in *G. m. morsitans*. Diversities were less in three laboratory cultures. Matings were random within populations. Populations were highly differentiated genetically. Populations were strongly subdivided, as indicated by fixation indices (F_{ST}) of 0.18 in *G. m. morsitans* and 0.17 in *G. m. submorsitans*. 35% of the genetic variance in *G. m. submorsitans* was attributed to differences between populations from The Gambia and Ethiopia. All available genetic evidence suggests that genetic drift is much greater than gene flow among *G. morsitans s.l.* populations.

Keywords

Glossina morsitans; gene flow; microsatellites; population genetics; tsetse flies; Africa

Introduction

Glossina (Glossina) morsitans sensu lato is the most widely distributed tsetse fly. Its distribution, however, is discontinuous and patchy, extending from Senegal in west Africa, easterly to the lowlands in western Ethiopia and southwards to Botswana, Zimbabwe and Mozambique. A disjunctive distribution is characteristic also of each subspecies, and is the result of historical forces involving climate and animal host distribution and abundance (Rogers, 1998).

The *G. morsitans* complex consists of three subspecies, *G. m. morsitans* Westwood, *G. m. centralis* Machado and *G. m. submorsitans* Newstead. The geographical distribution of subspecies is allopatric: *G. m. submorsitans* is distributed in the north below the Sahel, from Senegal to Ethiopia. *Glossina m. morsitans* occurs in Tanzania to Mozambique and eastern Zambia and Zimbabwe. *Glossina m. centralis* occurs in Angola, northern Botswana, Uganda, Zaire and western Zambia (Jordan, 1993). Laboratory breeding studies indicate that the subspecies cross freely in cages but there is post-mating reproductive isolation. Hybrid males are sterile and hybrid females are semi-sterile (Curtis, 1972; Gooding, 1990). Clearly these taxa have a common ancestor and share large regions of their genomes, but each has evolved different adaptations particularly to climate (Robinson *et al*., 1997a, b; Rogers, 2000).

Correspondence: Professor E. S. Krafsur, Entomology, Iowa State University, Ames, IA 50011-3222, U.S.A. E-mail: ekrafsur@iastate.edu.

Experiments using mark, release and recapture methods (Rogers, 1977) and analysis of population dynamics (Vale *et al*., 1984; Williams *et al*., 1992) indicate that *G. morsitans* is highly vagile. A strong propensity for dispersal predicts that gene flow among population clusters (i.e. among demes or subpopulations) would compensate for the genetic drift that characterizes small populations (Wright, 1978; Hartl & Clark, 1997). A high rate of gene flow makes gene frequencies among subpopulations more homogeneous. Study of the breeding structures of *G. morsitans s.l.* populations was carried out by analysis of variation in mitochondrial DNA via the single strand conformational polymorphism method (Black & DuTeau, 1997). The research indicated surprisingly high levels of genetic differentiation in *G. m. morsitans* (Wohlford *et al*., 1999), *G. m. submorsitans* (Krafsur *et al*., 2000) and *G. m. centralis* (Krafsur *et al*, 2001). Mitochondrial DNA is single-copy and inherited maternally. There is no recombination so the mitochondrial genome behaves as a single locus with many alleles. For the foregoing reasons, mitochondrial variation serves as a sensitive indicator of genetic drift and bottlenecks in population size (Avise, 1994). Indeed, history records bottlenecks in *G. morsitans* populations caused by the great rinderpest pandemic first recorded in sub-Saharan Africa in 1886 (Ford, 1971). An independent assay of genetic variation could help to confirm or deny the picture of highly differentiated populations among which there is little gene flow. Here we provide an independent assay of genetic variation in the same tsetse populations examined earlier. We use microsatellite loci to estimate the genetic parameters of diversity and gene flow. Microsatellites are short repetitive nucleotide sequences with conserved flanking regions that may be amplified by using the polymerase chain reaction. Microsatellite loci were recently developed in morsitans group flies (Baker & Krafsur, 2001).

Materials and methods

Sampling

Glossina m. submorsitans from The Gambia (five demes) and Ethiopia (two demes) were sampled by Drs Nigel Griffiths and Steve Mihok, as explained earlier (Krafsur *et al*., 2000). *Glossina m. morsitans* samples were from Kakumbi, Zambia, Changara, Mozambique, and Mana Angwa, Mana Pools, Rekomitjie and Makuti, all in Zimbabwe (Wohlford *et al*., 1999). The *Glossina m. centralis* were sampled in *Zambia* (Kasanka, Chunga), Namibia (Katima Mulilo) and Botswana (Kwando, Okavango). *Glossina m. morsitans* laboratory cultures were maintained at the International Centre for Insect Physiology and Ecology and sampled late in 1995. The culture originated in Kariba, Zimbabwe, in 1967 and was maintained at Langford, Bristol, U.K. (Jordan *et al*., 1970). Their history at ICIPE is confounded with supplementary stocks of various origins. The *G. m. submorsitans* laboratory culture was established from flies caught at the River Komoe, Burkina Faso (about 150km south-west of Bobo-Dioulasso) in late 1979 or early 1980 (Gooding, 1981). The culture was begun by Professor Ron Gooding with 25 puparia at the University of Alberta, where it was maintained at densities of 90–120 inseminated females for approximately 112 generations. It was selected for certain allozyme markers (Gooding, 1990). Assuming selective neutrality at diploid loci, it can be shown that the expected level of heterozygosity (diversity) with respect to the foundation stock is 0.61.

Processing samples and microsatellite amplifications

DNA was extracted from whole bodies by using a CTAB method (Shahjahan *et al*., 1995). The primers for micro-satellite loci were: *CAG133*, F 5′-ATT TTT GCG TCA ACG TGA-3′ and R 5′-ATG AGG ATG TTG TCC AGT TT-3′; *GmcCAG2*, F 5′-GCT TTT CTC GTC CCA TAA-3′ and R 5′-GCG TTG TTG ATGACT GTG-3′; *Gmm22*, F 5′-CGT AAA CGC GGG CTT GT-3′ and R 5′-CAA TTT GGC TGG CTG TCC-3′; *Gmm5B*, F 5′-GAA TTG TTA TGA GTG CAT GT-3′ and R 5′-ATG CGA CAC GAC ACA ATA AG-3′; *Gmm9B*, F 5′-TTT CCT ATA TTG CGA TTA-3′ and R 5′-CGT TTA CGT TAC CCA GAA-3′; *GmsCAG2*, F 5′-GCT TTT CTC GTC CCA TAA-3′ and R 5′-GCG TTG TTG ATG ACT GTG-3′ (Baker & Krafsur,

2001). PCR reactions consisted of 10 × PCR buffer, 0.4mM dNTP, 1.5mM $MgCl_2$, 0.5 μM each of forward and reverse primers, 0.5–1 μL of template DNA, and 0.25 U Biolase® polymerase for a final volume of 10 μL. The thermocycling profile was 35 cycles of 94°C for 40 s, 50°C for 40 s, and 72°C for 30 s. Amplifications were performed in a PTC-100 programmable thermal cycler (MJ Research, Waltham MA, U.S.A.).

Acrylamide gel electrophoresis and silver staining

After amplification, the PCR reactions were diluted 1:5 in a loading buffer consisting of 10mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol. The samples were denatured for 5min at 95°C just before acrylamide gel electrophoresis and then electrophoresed on a 5% denaturing polyacrylamide gel (32 × 50 cm × 0.4 mm thick) by using the Sequi-General GT Nucleic Acid Electrophoresis Cell (Bio-Rad). The gels were run at a constant 100 W and 50°C. After electrophoresis, the gels were fixed in 10% glacial acetic acid at I least 30 min and then silver stained by using the procedures of Black & DuTeau (1997). Alleles were scored according to their mobilities on polyacrylamide gels. Each allele was assigned a value relative to the same DNA bp standard by measuring their distances from the origin.

Data analysis

Chi-square contingency tests were used to test for homogeneity of allele frequencies among populations. Nei's (1987) unbiased estimator of gene diversity h_e at a locus is $2n(1-x_i^2)/(2n-1)$ where x is the frequency of allele i and n is the number of flies examined. This gives the expected heterozygosity when mating is random and other Hardy–Weinberg criteria apply. Diversity over r loci is $H_E = h_e/r$ with variance $(h_e-H_E)^2/[n(n-1)]$, where n is the number of flies in the sample.

F statistics were used to examine the goodness of fit of genotypic frequencies to Hardy–Weinberg expectations. The computational methods of Weir & Cockerham (1984) were used. The *F* statistics measure variance in allele frequencies and are appropriate for the infinite allele mutation model, but they may also be applied to loci in which the stepping stone mutation model applies (Gaggioti *et al*., 1999). Microsatellite loci fall somewhere between the two mutation models. ARLEQUIN 2.0 (Schneider *et al*, 2000) and FSTAT 2.9.3 (Goudet, 1995, 2001) were used to calculate most genetic statistics.

F statistics lead to estimates of gene flow in terms of the mean number of reproducing migrants per generation exchanged among populations, N_em, which was estimated by using several models. The private allele method of Slatkin (Slatkin & Barton, 1989) was used because allelic diversities were large, leading to overestimates of dispersal when based on *F* statistics.

Tests of selective neutrality included the Ewens–Watterson, Ewens–Watterson–Slatkin and Chakraborty's tests, which were accomplished by using the ARLEQUIN software. These tests are based on the infinite allele mutation model, which is not entirely appropriate for microsatellites. Chakraborty's test (Chakraborty, 1990) compares the number of alleles in a sample with the number expected in a stationary population at mutation-drift equilibrium.

The frequency of presumptively null alleles (caused by mutations at annealing sites) was estimated according to a maximum likelihood method in which only null heterozygotes are scored (Brookfield, 1996). The chief assumption is that any heterozygote deficiencies were caused only by null alleles.

The software BOTTLENECK vs. 1.2 (Piry *et al*., 1999) was used to determine if the microsatellite diversities confirmed historical evidence of a severe and prolonged bottleneck in *G. morsitans* populations (Ford, 1971; Rogers, 1995).

Results

GmsCAG29 oligonucleotide primers failed to amplify Changara *G. m. morsitans* DNA. *Gmm22* primers did not amplify in Changara and Kakumbi *G. m. morsitans*. These results are presumptive evidence of fixed differences at annealing sites in these populations. Allelic frequencies at the amplified loci are available from E.S.K. by e-mail.

Mutation-drift equilibrium tests

Ewens–Watterson and Ewens–Watterson–Slatkin tests of selective neutrality and mutation-drift equilibrium were nonsignificant. Chakraborty's test of population amalgamation indicated a significant excess of alleles in *G. m. submorsitans* in Chankar, Kudang and Kunting, all in The Gambia, and *G. m. morsitans* in Changara, Mozambique. An excess of alleles can arise when the sampled populations have arisen by fusion of two or more previously isolated populations and this is consistent with field studies in *G. m. submorsitans* (Rawlings *et al*., 1993). The foregoing tests support the hypothesis that tsetse populations were at, or close to, mutation-drift equilibrium.

Microsatellite diversities in populations

Population diversities (heterozygosities) are summarized in Table 1. Mean alleles per locus varied from 3.3 in a laboratory culture of *G. m. submorsitans* to 7.5 in the Kunting sample. The variation in means differed significantly among the natural populations in both subspecies (Table 1). Averaged over loci, most heterozygosities exceeded 50%. Only three loci amplified in the Changara *G. m. morsitans*, thereby accounting for the least diversities observed in wild populations. Heterozygosities in three laboratory cultures were about the same as those observed in natural populations, testifying to the careful husbandry necessary to maintain tsetse in culture (Gooding *et al*, 1997). However, allelic diversity in laboratory cultures was less than in wild populations, as may be expected by drift in closed populations (Maruyama & Fuerst, 1985). Heterozygosity in the Burkina Faso laboratory culture was 0.54 compared with the mean 0.69 for wild samples, about 78% versus the theoretical expectation of 58%. Allelic diversity in the Burkina Faso laboratory culture, by contrast, was 3.37/6.97, or 48% of the mean value for wild flies.

Chi-square tests of homogeneity of allele frequencies in *G. m. submorsitans* indicated that populations differed significantly in The Gambia ($\chi^2_{(272)} = 997$, $P << 0.001$), in Ethiopia ($\chi^2_{(43)} = 195.6$, $P << 0.001$), and therefore over all populations ($\chi^2_{(420)} = 2022$, $P << 0.001$). In *G, m. morsitans*, allele frequencies differed among populations at each locus ($\chi^2_{(56)} = 1452.6$, $P << 0.001$). Clearly, tsetse populations were genetically differentiated.

Contrasts between expected and observed heterozygosities (Table 1) are a measure of departures from Hardy–Weinberg equilibria, and this is best expressed by using *F* statistics. F_{IS} in Table 1 is a measure of departures from random mating in populations. Its expectation is $-2N^{-1}$ (Weir, 1996) and the expected value, for samples of *c*. 24, is −0.02. Only *G. m. submorsitans* from the River Ketto showed a significantly large gap between expected and observed heterozygosities. The cause was not inbreeding, but the occurrence of null alleles. The paucity of heterozygotes at River Ketto occurred at *Gmm8, GmsCAG29, GmcCAG2* and *Gmm22*. Estimated frequencies of null alleles were 0.13, 0.38, 0.24 and 0.26, respectively, assuming the heterozygote deficiencies were caused only by null alleles.

Single locus statistics and genetic differentiation within and among natural populations

The number of alleles per locus varied from six in *GpCAG133* to 21 in *Gmm22* (Table 2). Averaged over only the wild populations, heterozygosities (H_E) varied from 0.585 at *GpCAG133* to 0.915 at *Gmm5B*.

F_{IS} measures departures from random mating within populations (Table 2) and a significant departure was observed only at *Gmm22* in *G. m. submorsitans*. Estimates averaged over loci, however, indicated that matings within populations were random. In contrast, departures from random mating among populations were substantial. Averaged over loci, F_{ST} was 0.166 in *G. m. submorsitans* and 0.185 in *G. m. morsitans*. The comparable estimate in *G. m. centralis* was 0.186 (Krafsur *et al*., 2001). All the foregoing estimates were significantly different from zero. Nei's (1987) analogous statistic G_{ST} was 0.168 in *G. m. submorsitans*, 0.181 in *G. m. morsitans* and 0.185 in *G. m. centralis*. Analyses of variance, presented later, gave closely similar estimates.

Microsatellite diversities in subspecies

Mean heterozygosities varied from 0.70 in *G. m. centralis* (Krafsur *et al*., 2001), 0.727 in *G. m. morsitans*, to 0.808 in *G. m. submorsitans*. These mean heterozygosities differed significantly when tested by ANOVA ($F = 104.3$, d.f. = 2 and 447, $P < 0.001$). There was, however, little difference in the allelic frequency distributions among subspecies, so they were combined in Fig. 1. The frequency of most (54%) alleles varied between 1 and 10%.

Seventy-six alleles were detected among six loci in *G. m. submorsitans* and 55 alleles among five loci in *G. m. morsitans* (Table 3). Fifty-three alleles among six loci were recorded *G. m. centralis* (Krafsur *et al*., 2001). The overall mean number of alleles per locus was 11 in *G. m. morsitans*, 12.7 in *G. m. submorsitans* and only 8.8 in *G. m. centralis*. These means differed significantly when tested by ANOVA ($F = 18.8$, d.f. = 2 and 447, $P < 0.001$). The proportions of private alleles (alleles unshared among populations) were homogeneous among subspecies ($\chi^2_{(2)} = 3.09$, $P \approx 0.21$) and averaged 16%. The frequency of private alleles, averaged over subspecies, was 0.082, leading to an average level of gene flow $Nm = 0.90$ and corresponding mean $F_{ST} = 0.22$. Nm and the corresponding F_{ST} values for each subspecies are included in Table 3.

Analysis of variance in allele frequencies

In *G. m. morsitans*, 18% of the variance in allele frequencies was among the six populations, and 82% lay within populations (Table 4). $F_{ST} = 0.182$, closely agreeing with the other estimates of this parameter. The variance components, hence the F statistics, all differed significantly from zero.

In a hierarchical classification, *G. m. submorsitans* populations were assigned to one of two countries, and the variance in allele frequencies partitioned accordingly (Table 6). About 34% of the variance was attributed to countries and 65% lay within populations. Only a trivial 1% was attributed to populations within countries. F_{CT} at 0.338, reflects the wide separation between easternmost and westernmost *G. m. submorsitans* and hardly differs from the F_{ST} estimate of 0.348.

Gene flow among populations

In *G. m. submorsitans*, pairwise estimates of F_{ST} varied essentially from zero to 0.06 within countries, and from 0.23 to 0.38 between countries (Table 6). The corresponding levels of dispersal N_m between countries was much less than one, predicting further differentiation by drift. Migrants within countries, however, were substantially above the 'critical' value of one (Wright, 1978). Pairwise estimates of F_{ST} in wild *G. m. morsitans* varied from 0.034 to 0.328 (Table 7). Mana Angwa vs. Makuti and Changara vs. Kakumbi were particularly low, suggesting high rates of gene flow. Gene flow between the other pairs were near unity or less. The laboratory cultures were more closely related to each other than to any particular wild sample.

Discussion

Diversity estimates for Changara *G. m. morsitans* were flawed because two loci did not amplify. Amplification of two of four mitochondrial loci failed in the same samples (Wohlford *et al*., 1999), raising the possibility of fixed mutant annealing sites. The Changara population may therefore be more genetically differentiated than indicated by the available data and may, indeed, belong to a separate fly belt (Ford, 1971).

Allelic frequencies differed significantly among populations in both *morsitans* subspecies, indicating that gene flow was insufficient to overcome the force of genetic drift. Three possibilities can explain this genetic differentiation: (1) rates of dispersal were low; (2) strong and different selection regimes operate locally, and (3) populations were not in mutation-drift equilibrium. A transient disequilibrium would obtain as tsetse populations recovered from their earlier declines in density and range caused by the rinderpest epizootic of 1889–1896 (Ford, 1971). We were unable, however, to refute null hypotheses of mutation-drift equilibrium in our samples. Nevertheless, there were more alleles than expected, given the observed levels of heterozygosity in *G. m. submorsitans* from The Gambia. Coalescence of expanding populations can cause such a distribution (Chakraborty, 1990).

Gene diversities were substantial at all loci in *G. morsitans s.l.*, testifying to historically abundant population sizes or remarkably high mutation rates. The microsatellite data offer no compelling evidence of earlier severe bottlenecks in subspecies. If mutation rates were particularly high, populations would remain at mutation-drift disequilibrium because only *c*. 800 generations had elapsed since the end of the rinderpest epizootic of 1889–1896 and sampling in 1996. Microsatellite loci in humans may show mutation rates as much as 10^{-3}–10^{-4}, although rates in *Drosophila* species are much less, *c*. 6×10^{-6} (Schug *et al*., 1997). Recovery from putative bottlenecks may be assumed to have been slow because the rapidity of mammalian host recovery from the rinderpest would have been a strongly limiting factor in tsetse population recovery and growth. Tsetse have low reproductive rates, with a population doubling time of *c*. 46 days, given a daily rate of increase of 1.5%. The historical record shows range expansion in *G. m. morsitans* and *G. m. centralis* of approximately 7 km/year (Ford, 1971; Williams *et al*, 1992), but data seem to be lacking for *G. m. submorsitans*. Thus, the great diversities in *G. morsitans* microsatellite loci would seem to indicate that many tsetse flies survived the rinderpest epizootic, perhaps as numerous small, scattered populations, largely undetected in southern Africa.

Departures from random mating within populations (F_{IS})

Observed heterozygosities were usually consistent with those expected on Hardy–Weinberg criteria. Averaged over loci, mating was random in populations ($F_{IS} \approx 0$), with one exception. A large deficiency of heterozygotes was recorded at four of six loci in River Ketto *G. m. submorsitans*. In addition, when averaged over the wild *G. m. submorsitans* populations, *Gmm22* deviated significantly from Hardy–Weinberg genotypic proportions. The cause of these departures from expectation had nothing to do with inbreeding because all loci selectively neutral loci would have been affected. There are alternative possibilities. One is that heterozygote deficiency was caused by a failure to achieve adequate resolution on gels to distinguish heterozygotes from homozygotes. A more likely explanation is the occurrence of mutant annealing sites associated with the problematic loci. Such mutations highlight a common problem with microsatellites, which is that the locus includes all amplified nucleotides plus the annealing sites, not just the primary sequence repeat. Thus, a distinct allele may represent a polymorphism that occurs at interstitial regions between primer annealing sites and the primary repeat itself.

Departures from random mating among populations (F_{ST})

In contrast to F_{IS}, mean F_{ST} estimates all differed significantly from zero, leading to the conclusion that genetic drift was much more potent than dispersal among populations. Of course, matings are far more likely within than between populations, but it takes very little gene flow to prevent much drift. For this reason, F_{ST}s of ≥ 0.05 are generally considered to be evolutionarily significant (Wright, 1978). An estimate of 0.166 in *G. m. submorsitans* and 0.185 in *G. m. morsitans* compares with 0.186 in *G. m. centralis* (Krafsur *et al*., 2001). These estimates can be viewed as correlations between uniting gametes in populations relative to correlations between randomly chosen gametes in the population at large. The values are substantial, indicating large, and very comparable, measures of genetic drift in *G. morsitans sensu lato*. But they are underestimates because mean F_{ST} estimates were biased downward by high allele numbers. As heterozygosities approach unity, F approaches zero because $F = 1 - H_O/H_E$, where H_O is the observed and H_E is the expected heterozygosity. Clearly, F cannot be greater than levels of homozygosity (Hedrick, 1999). Moreover, high mutation rates also depress F_{ST} estimates, leading to overestimates of gene flow.

Analyses of variance

The percentage of genetic variation among *G. m. morsitans* populations was 18.2%. A similar value (17.7%) was obtained in *G. m. centralis* (Krafsur *et al*., 2001). Hierarchical ANOVA in *G. m. submorsitans* showed that 33.8% of variation was between the two countries sampled and only 1 % among populations within countries. The corresponding F_{ST} estimates were 0.182 in *G. m. morsitans*, 0.177 in *G. m. centralis* and 0.35 in *G. m. submorsitans*. The latter estimate is double that afforded by the Weir and Cockerham method.

Gene flow

As measures of gene flow, the foregoing F_{ST}s in *G. m. morsitans* and *G. m. centralis* indicate a mean level of exchange of about 1.14 reproducing flies per population per generation, according to Wright's island model. In *G. m. submorsitans*, equivalent gene flow among all sampled populations was 1.26, and between Ethiopia and The Gambia it was 0.46 reproducing flies per generation. Estimates of gene flow based on private allele frequencies (Table 3) may be contrasted with estimates based on frequencies from all alleles (Tables 2, 4 and 5). The private allele method can furnish less biased estimates where heterozygosities are large. Private alleles indicated that gene flow in *G. m. morsitans* and *G. m. centralis* was less than one reproducing migrant per generation, thereby predicting that drift will lead to fixation of alternative alleles (Wright, 1978). The corresponding values of N_em and F_{ST} in *G. m. submorsitans* were considerably less than estimates provided by analysis of heterozygosities.

Concluding remarks

The chief results of the research are twofold: there is much microsatellite diversity in *G. morsitans s.l.* and the variation is highly partitioned geographically. Thus, gene flow seems to be greatly restricted and this is consistent with all available genetic evidence, including chromosomal (Jordan *et al*., 1977; Southern, 1980), allozyme (Krafsur *et al*., 1997), mitochondrial (Wohlford *et al*., 1999; Krafsur *et al*., 2000) and microsatellite (Krafsur *et al*., 2001) polymorphisms. The chief question remaining is how to rationalize the picture of gene flow obtained by indirect means with that afforded by mark, release and recapture methods (Rogers, 1977), field sampling (Vale *et al*., 1984; Brightwell *et al*., 1992; Williams *et al*., 1992) and the historical record of *G. morsitans* recolonizing lands from which it presumably disappeared during the rinderpest (Ford, 1971).

Acknowledgements

Thanks to Nigel Griffiths and Steve Mihok for collecting most of the tsetse flies and Ron Gooding for the gift of *G. m. submorsitans* from his laboratory culture. Research was supported in part by USPHS grant no. AI40048. This is Journal Paper no. 19821 of the Iowa Agricultural and Home Economics Experiment Station, Project 3447 and supported in part by Hatch Act and State of Iowa funds.

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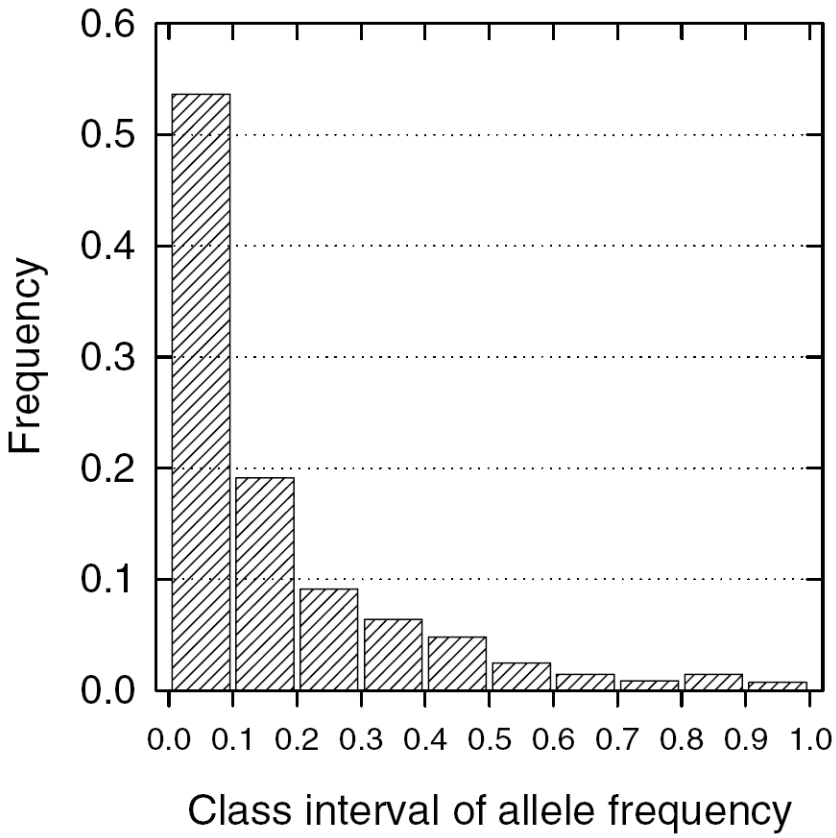

Fig. 1.
Distribution of allelic frequencies in *G. morsitans sensu lato*.

Table 1

Population diversities (heterozygosities) averaged over microsatellite loci in two *Glossina morsitans* subspecies.

Population	Mean sample size per locus	Mean no. of alleles per locus	Mean heterozygosity		F_{IS}[a]
			Observed	Expected	
G. m. morsitans: 5 loci[b]					
Lab A	19.4±1.6	3.6±0.7	0.424±0.119	0.394±0.118	−0.076
Lab B	18.2±2.5	4.8 ± 1.1	0.570 ±0.062	0.613 ±0.059	0.070
Changara	14.0 ±5.3	3.4±1.3	0.332±0.153	0.345±0.156	0.038
Kakumbi	14.2±3.3	4.2±1.3	0.528 ±0.141	0.493 ±0.136	−0.071
Makuti	21.2±1.5	6.0±1.8	0.535±0.137	0.551 ±0.123	0.029
Rekomitjie	23.6 ±0.2	6.6±1.9	0.578 ±0.099	0.587±0.115	0.015
Mana Angwa	22.6 ±1.2	6.2±1.7	0.621 ±0.091	0.590 ±0.076	−0.053
Mana Pools	22.6 ±1.2	7.2±1.1*	0.620 ±0.072	0.718 ±0.027	0.136
G. m. submorsitans: 6 loci[c]					
Lab (B. Faso)	24.0 ±0.0	3.3±0.7	0.590±0.129	0.539±0.112	−0.095
Dankuku	23.3 ±0.2	7.0±1.5	0.676±0.122	0.647±0.112	−0.045
Kunting	20.8 ±1.6	7.5±1.3	0.732±0.081	0.720 ±0.068	−0.017
Kenaba	22.7 ±0.7	7.3 ±1.7	0.676±0.113	0.694 ±0.094	0.026
Kudang	24.0 ±0.4	7.2±0.2	0.637 ±0.097	0.654±0.106	0.026
Bansang	19.3 ±0.4	7.3±2.3	0.705±0.143	0.669±0.142	−0.054
R. Ketto	22.8 ±1.0	6.7±1.6	0.473 ±0.088	0.723 ±0.054	0.346[d]
Chankar	22.7 ±0.6	5.8±0.8	0.762 ±0.029	0.726 ±0.039	−0.050

[a] $F_{IS} = 1 - HO/H_E$. Significance tests by randomized permutation.

[b] Alleles per locus in natural populations differ (ANOVA: $F = 72.4$, d.f. = 5, 585, $P < 0.001$).

[c] Alleles per locus in natural populations differ (ANOVA: $F = 21.0$, d.f. = 6, 967, $P < 0.001$).

[d] $P \approx 0.0002$ for randomization tests with larger F than observed.

Table 2

Single-locus heterozygosities (H_E) and F statistics by the method of Weir and Cockerham in field collected *G. m. morsitans* and *G. m. submorsitans*.

Locus	No. alleles	H_E[a]	F_{IS}	F_{ST}	F_{IT}
G. m. submorsitans					
Gmm5B	20	0.910	−0.015	0.064	0.051
Gmm8	8	0.740	0.086	0.071	0.151
GmsCAG29	11	0.848	0.027	0.080	0.105
GmcCAG2	7	0.705	−0.059	0.232	0.187
GpCAG133	9	0.723	0.010	0.532	0.537
Gmm22	21	0.921	0.117	0.065	0.174
Means[a]	12.67	0.808	0.035	0.166	0.195
SE	6.22	0.040	0.029	0.075	0.069
	20	0.915	−0.015	0.064	0.051
G. m. morsitans					
Gmm5B	20	0.915	−0.015	0.064	0.051
Gmm8	7	0.658	−0.021	0.257	0.242
GmsCAG29	10	0.799	−0.054	0.265	0.226
GpCAG133	6	0.585	0.013	0.315	0.324
Gmm22	12	0.678	0.025	0.125	0.12
Means[a]	11.00	0.727	0.029	0.185	0.206
SE	5.57	0.058	0.030	0.054	0.032

[a] Expected unbiased heterozygosity (Nei, 1987) over all populations.

[b] Jackknife means and standard errors for F statistics.

Table 3

Microsatellite private alleles, departures from random mating among populations F_{ST}, and average number of reproducing migrants per generation *Nm* according to the island model in *Glossina morsitans* subspecies.

Taxon	No. populations	No. loci	No. alleles	Private alleles[a]	Freq. private alleles	*Nm*[c]	F_{ST}[d]
G. m. morsitans	6	5	55	12	0.097	0.68	0.27
G. m. centralis	7	6	53	5	0.095.	0.70	0.26
G. m. submorsitans	7	6	76	13	0.055	1.81	0.12

[a] Private alleles among taxa: $\chi^2_{(2)} = 3.09$, $P \gg 0.21$.

[c] Average number of reproducing migrants per generation, estimated by the private allele method of Slatkin & Barton (1989).

[d] $F_{ST} \gg (4Nm+1)^{-1}$.

Table 4

Analysis of variance in allele frequencies in *G. m. morsitans* by the method of Excoffier *et al*. (1992).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	61.578	0.24434	18.22
Within populations	270	296.201	1.09704	81.78
Total	275	357.779	1.34138	

Fixation Index $F_{ST} = 0.182$, $P < 0.001$.

Table 5

Analysis of variance in allele frequencies in *G. m. morsitans* by the method of Excoffier *et al*. (1992).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*F* statistics	Significance
Among countries	1	12.273	0.10833	33.78	F_{CT} = 0.338	< 0.0001
Among populations within countries	5	1.709	0.00333	1.04	F_{SC} = 0.016	< 0.022
Within populations	275	57.486	0.20904	65.18	F_{ST} = 0.348	< 0.0001
Total	281	71.468	0.32070			

Table 6

Pairwise genetic distance measures for *G. m. morsitans* in terms of F_{ST} in upper diagonal and equivalent dispersal rates N_m in the lower diagonal.

	Bansang	Kenaba	Kunting	Kudang	Dankunku	R. Ketto	Chankar
Bansang	3/4	0.054	−0.019	0.021	0.065	0.251	0.242
Kenaba	4.35	3/4	0.047	0.031	0.014	0.383	0.347
Kunting	∞	5.05	3/4	0.008	0.052	0.238	0.232
Kudang	11.82	7.84	30.55	3/4	0.059	0.231	0.228
Dankunku	3.58	18.06	4.57	3.98	3/4	0.415	0.370
R. Ketto	0.74	0.40	0.80	0.83	0.35	3/4	0.003
Chankar	0.78	0.47	0.82	0.85	0.42	85.67	3/4

Table 7

Pairwise genetic distance measures for *G. m. morsitans* in terms of F_{ST} in upper diagonal and equivalent dispersal rates N_m in the lower diagonal.

	Lab A	**Lab B**	**Rekomitjie**	**M.Pools**	**M.Angwa**	**Kakumbi**	**Changara**	**Makuti**
Lab A	—	0.1058	0.2133	0.3283	0.2334	0.2561	0.2915	0.2522
Lab B	2.11	—	0.1998	0.2322	0.1944	0.1976	0.2235	0.2248
Rekomitjie	0.92	1.00	—	0.1948	0.1438	0.1186	0.2869	0.1318
M. Pools	0.51	0.83	1.03	—	0.1957	0.2088	0.2917	0.2521
M. Angwa	0.82	1.04	1.49	1.03	—	0.2207	0.2572	0.0336
Kakumbi	0.72	1.02	1.86	0.95	0.88	—	0.0587	0.2445
Changara	0.61	0.87	0.62	0.61	0.72	4.01	—	0.3122
Makuti	0.74	0.86	1.65	0.74	7.19	0.77	0.55	—