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Population structure of the tsetse fly Glossina pallidipes estimated by allozyme, microsatellite and mitochondrial gene diversities

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Diversities at nuclear and mitochondrial loci were examined in eleven natural populations of Glossina pallidipes from east and southern Africa. Alleles in each class of loci are assumed to be selectively neutral. Allozyme gene diversities (heterozygosities) averaged over eight loci were 0.146 among seven Kenya populations and 0.201 among four southern African populations. Microsatellite diversity averaged over three loci was 0.250 in Kenya and only 0.218 in southern Africa. Mitochondrial diversities averaged 0.504 in Kenya and only 0.156 in southern Africa. Mitochondrial and microsatellite diversities in the populations were strongly correlated with each other, but uncorrelated with allozyme diversities. In contrast to the allozyme diversities, mitochondrial and microsatellite variation indicated a severe and prolonged reduction in population size in southern Africa. Genetic distances among populations increased with the geographical distances between them. Allozyme diversities in southern populations were conserved. Genetic differentiation at allozyme loci among populations was greatly damped when compared with the other markers. The foregoing can be explained if allozyme diversities were maintained by balancing selection. Three main points emerged: genetic data confirm the historical evidence that southern G. pallidipes populations experienced a severe and prolonged bottleneck; allozyme variation was conserved in the bottlenecked populations; and gene flow among populations is surprisingly restricted.

Keywords

Allozymes, breeding structure, Glossina pallidipes, mitochondrial DNA, single strand conformational polymorphisms, microsatellites, balancing selection

Comments

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Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities

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Abstract

Diversities at nuclear and mitochondrial loci were examined in eleven natural populations of *Glossina pallidipes* from east and southern Africa. Alleles in each class of loci are assumed to be selectively neutral. Allozyme gene diversities (heterozygosities) averaged over eight loci were 0.146 among seven Kenya populations and 0.201 among four southern African populations. Microsatellite diversity averaged over three loci was 0.250 in Kenya and only 0.218 in southern Africa. Mitochondrial diversities averaged 0.504 in Kenya and only 0.156 in southern Africa. Mitochondrial and microsatellite diversities in the populations were strongly correlated with each other, but uncorrelated with allozyme diversities. In contrast to the allozyme diversities, mitochondrial and microsatellite variation indicated a severe and prolonged reduction in population size in southern Africa. Genetic distances among populations increased with the geographical distances between them. Allozyme diversities in southern populations were conserved. Genetic differentiation at allozyme loci among populations was greatly damped when compared with the other markers. The foregoing can be explained if allozyme diversities were maintained by balancing selection. Three main points emerged: genetic data confirm the historical evidence that southern *G. pallidipes* populations experienced a severe and prolonged bottleneck; allozyme variation was conserved in the bottlenecked populations; and gene flow among populations is surprisingly restricted.

Keywords

Allozymes; breeding structure; *Glossina pallidipes*; mitochondrial DNA; single strand conformational polymorphisms; microsatellites; balancing selection

Introduction

Tsetse flies (Diptera: Glossinidae) are confined to Africa south of the Sahara. Females are larviparous, and well-fed flies require at least 14 days to mature the first larva, which pupariates soon after larviposition. Thereafter a larva may be produced at successive 10-day intervals. Generation time is long. Thus tsetse flies have a low fecundity and great longevity. Small changes in pupal and adult survival rates have large effects on population size. Populations are regulated by strong density-dependent mechanisms and are highly resilient (Rogers & Randolph, 1984, 1985). Tsetse flies of both sexes are exclusively blood-feeders and are the only known biological vectors of human and animal trypanosomiasis. Only thirty-two species and subspecies are known, and *G. pallidipes* is one of the most economically important species.

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G. pallidipes has a wide but discontinuous distribution in east and southern Africa (Ford, 1971).

Investigation of the breeding structure of *Glossina pallidipes* using allozymes indicated surprisingly high levels of genetic differentiation among the eleven populations examined (Krafsur *et al.*, 1997). Thirty alleles segregated at eight polymorphic loci. Phosphoglucose isomerase (*Pgi*) was the most diverse locus with six alleles, and 68% of the tsetse flies were heterozygous at this locus. The most useful measure of genetic differentiation is F_{ST}, which measures departures from random mating among populations; F_{ST} is the correlation of two randomly chosen genes in a sub-population relative to the correlation in the population as a whole. Measured at allozyme loci by the method of Weir & Cockerham (1984), F_{ST} in *G. pallidipes* was 0.24 ± 0.05, which estimates an equivalent average exchange rate of only 0.8 reproducing flies per population per generation, a low rate of gene flow that predicts further differentiation by drift. Further investigation, using single-strand conformational polymorphisms in mitochondrial DNA, indicated virtually the same level of gene flow among eighteen *G. pallidipes* populations (Krafsur & Wohlford, 1999).

Of particular interest was the contrast in gene diversities between mitochondrial (Krafsur & Wohlford, 1999) and nuclear (Krafsur *et al.*, 1997) gene loci among southern African populations. Southern *G. pallidipes* showed abundant allozyme diversity, but a paucity of mitochondrial variation. Populations in Kenya and Ethiopia showed abundant mitochondrial variation. Moreover, *G. morsitans centralis* populations in southern Africa were also depauperate of detectable mitochondrial variation (Krafsur *et al.*, 2001) but considerable variation was demonstrated among *G. morsitans s.l.* from elsewhere in Africa (Wohlford *et al.*, 1999; Krafsur *et al.*, 2000). History explains the low mitochondrial diversity in southern Africa: a rinderpest pandemic hit southern Africa in 1887 with particular ferocity, causing about 90% mortality of a wide range of mammals that included tsetse primary hosts and which had demonstrable, long-lasting effects on tsetse fly distribution and abundance (Ford, 1971). Allozyme diversity in southern Africa *G. pallidipes* was, however, greater than in Kenyan populations, an observation that could be taken as empirical evidence of natural selection favouring heterozygosis, i.e. balancing selection (Kreitman & Akashi, 1995; Eanes, 1999; Black *et al.*, 2001). Heterozygosities at loci coding for phosphoglucoisomerase and malate dehydrogenase were found to be greater in a laboratory culture than in two samples from field populations (Nesbitt *et al.*, 1990). The contrast of mitochondrial and nuclear gene diversities in *G. pallidipes* invites further investigation. Selectively neutral markers are required in the nuclear genome. Here I make explicit comparisons between inferences on breeding structure evaluated by microsatellite, allozyme and mitochondrial genetic variation.

Results

Microsatellite allele frequencies and estimates of breeding structure

Six alleles were scored at *GpCAG133*, seven at *GmcCA16C*, and five at *GmsCAG2*. Of these eighteen alleles, three were confined to a single population for a frequency of 0.167 ± 0.088 private alleles. Mean heterozygosities (diversities) varied nearly eightfold between the three loci and the mean over all loci and populations was 0.332 (Table 1). The allele frequencies are given in the appendix and differed significantly among the eleven populations at *CAG133* ($\chi^2_{50} = 582$, $P \ll 0.001$), *CA16C* ($\chi^2_{60} = 125$, $P \ll 0.001$) and *CAG2* ($\chi^2_{40} = 71.9$, $P \approx 0.001$). Random mating was indicated within populations (F_{IS}) but not between them (F_{ST}, Table 1). The degree of among-population differentiation (F_{ST}) varied inversely with heterozygosity. An estimate of F_{ST}, based on the frequency of private alleles (Slatkin & Barton, 1989) was 0.474. The corresponding Weir and Cockerham estimate of F_{ST} was 0.33 (Table 1).

Allozyme, microsatellite and mitochondrial diversities compared

For each population, diversities averaged over the nuclear loci are compared with the mitochondrial diversities in Table 2. Because mitochondrial loci are in absolute linkage disequilibrium, each combination of alleles was designated a haplotype, and nineteen such haplotypes were observed. Averaged over the same eleven populations, mean haplotype diversity was 0.385 ± 0.306, allozyme diversity was 0.166 ± 0.037, and microsatellite diversity was 0.238 ± 0.071. Both the mean and the variance were greater at mitochondrial loci and the coefficient of variation was nearly threefold the values at genomic loci. Allozyme diversities were independent of microsatellite (Spearman rank order correlation $r = 0.15$, $P = 0.6$) and mitochondrial variation ($r = -0.12$, $P = 0.71$, Fig. 1A), but the mitochondrial and microsatellite diversities were correlated with each other $r = 0.74$, $P = 0.007$, Fig. 1B, as they should be if the variation were selectively neutral.

Pair-wise ($n = 55$ pairs) Cavalli-Sforza & Edwards chord genetic distances for mitochondrial and nuclear loci were compared using Mantel tests. Strong correlation coefficients were obtained for each of the three comparisons ($r \approx 0.60$–0.64, $P < 0.001$). Congruencies among the three measures suggest that mitochondrial, allozyme and microsatellite gene flow followed the same basic pattern. The slopes of regression differed however. The slope of mitochondrial distance on microsatellite distance was 0.98, indicative of a 1 : 1 correspondence in these genetic distance measures; but the slopes of mitochondrial and microsatellite genetic distances regressed on the allozyme distance were 2.1 and 1.7, respectively. Thus, pair-wise allozyme genetic distances between populations averaged half the distances of the neutral genetic markers.

The same pair-wise genetic distance measures were regressed on the geographical distances between populations (Fig. 2). Mitochondrial genetic distances were greater than the corresponding nuclear measures. The slopes of microsatellite and mitochondrial regressions were congruent and 3.45-fold greater than the slope of the allozyme regression. The shallow slope of allozyme genetic distances regressed on geographical distance is further evidence of balancing selection.

Cluster analysis of the genetic distance measure on allozyme loci showed two principle groups, Kenya and southern Africa (Fig. 3A). The mitochondrial dendrogram (Fig. 3B) was much deeper than the allozyme and microsatellite dendrogram; it also showed two deeply rooted clusters corresponding to Kenya and southern Africa, but Changara clustered most closely with Dakabuko and Alangoshira, populations in Eastern Kenya. Microsatellite loci showed the same north–south bifurcation as the allozyme loci (Fig. 3C).

Regional contrasts

Allozyme diversities were greater among the southern populations than among the Kenya samples. On the other hand, microsatellite and mitochondrial diversities were less in southern Africa than in Kenya and the differences were highly significant (Table 3). Most of the mitochondrial diversity in southern Africa was in the Changara sample. Mitochondrial diversity among the three Zimbabwean samples was only 0.055, but allozyme diversity was 0.202.

ANOVA and hierarchical estimates of gene flow

Analyses of variance of gene frequencies and haplotype frequencies are shown in Table 4. In all classes of genetic variation, about two-thirds of the total variation was attributed to populations nested in regions. Total variance at the mitochondrial loci was more than twice the variance at nuclear loci.

Fxy (Table 4) may be interpreted as the correlation of alleles or haplotypes in two randomly chosen flies in sampling unit *x* (population or region) relative to the correlation of two randomly chosen flies in sampling unit *y* (region or the total). *Fxy* for populations-total is an estimate of F_{ST}. If mating is random then *Fxy* at nuclear loci will not differ significantly from zero. The data indicate departures from random mating or dispersal among populations at all levels of hierarchy. The *F*-values were used to make estimates of the equivalent numbers of reproducing migrants *Nm* (Table 4) among populations, thereby providing an indirect measure of actual gene flow. Mitochondrial and microsatellite *F* statistics indicated more genetic differentiation than estimates based on allozyme loci. Thus allozyme loci underestimated the differentiation, which leads to overestimates of gene flow.

Discussion

Microsatellites in G. pallidipes

Two of the three microsatellite loci used here were isolated from *G. morsitans* (Baker & Krafsur, 2001). Seven additional *G. morsitans* microsatellite loci amplified *G. pallidipes* DNA, but were unsuitable for scoring genotypes accurately. Microsatellites are selectively neutral and generally show greater diversities than allozyme loci. Available reports suggest that microsatellites may not be greatly abundant in tsetse fly genomes: three polymorphic loci have been isolated from *G. palpalis sensu lato* by Solano *et al.* (1997), and 13 were developed by Luna *et al.* (2001). Baker & Krafsur (2001) reported on 14 polymorphic loci in *G. morsitans sensu lato*. In *G. pallidipes*, allele frequencies at each microsatellite locus differed significantly among populations and each indicated that mating was random within, but not among, populations. *CAG133* indicated much greater genetic differentiation than *CA16C* and *CAG2*, a consequence of its greater diversity. The mean interpopulation estimate of differentiation, F_{ST}, was 0.33. An independent estimate from the same data of $F_{ST} = 0.47$ was afforded by Slatkin and Bar's (1989) method, based on the frequency of private alleles. The coefficient of variation of the private allele frequency (0.167 ± 0.088) was *c.* 50%, so the F_{ST} estimate would not differ significantly from the estimate provided by Weir & Cockerham's (1984) method.

Classes of genetic variation compared

Examination of three classes of genetic variation in the same *G. pallidipes* populations leads to three principal conclusions: (i) Indices of genetic differentiation among populations were substantial and seemingly greater than that suggested by well conducted ecological studies; (ii) a stringent and prolonged bottleneck in population size was detected in southern African populations; and (iii) allozyme variation was conserved in the bottlenecked populations.

Estimated population diversities were least at allozyme loci and greatest at the mitochondrial loci. Electrophoresis detects only about one-third of amino acid substitutions in proteins (Lewontin, 1974; Avise, 1994), but the level of resolution afforded by allozyme variation is not an issue here. Mitochondrial and microsatellite diversities in populations were correlated with each other but were independent of allozyme variation. Selectively neutral variation should covary among equilibrium populations but allozyme heterozygosities did not.

The positive slopes of three classes of pair-wise genetic distances regressed on the corresponding geographical distances validates them all as estimators of gene flow, but the slope of allozyme genetic distances was about one-third of the mitochondrial and microsatellite slopes. Clearly, genetic distance was correlated with geographical distance between populations. The congruency of mitochondrial and microsatellite variation indicates that female tsetse flies disperse at roughly the same rate as the males. Vale *et al.* (1984) showed that the females dispersed at the greater rate by using mark, release and recapture methods, but

the timescale and resolution of the indirect genetic and the direct experimental approaches are not readily comparable.

The pair-wise regressions of genetic distance indicate that the effects of genetic drift at allozyme loci were significantly dampened. Simulations show that the balancing selection in subdivided populations reduces F_{ST} (Schierup *et al.*, 2000). Among 144 isofemale lines of *D. melanogaster* representing 18 locations worldwide, allozyme and mitochondrial diversities and distance measures were uncorrelated, and on this basis it was suggested that selection acting on allozyme profiles explained the independence of the two genetic measures (Hale & Singh, 1991).

Mitochondrial variation in *G. pallidipes* populations varied from three to eightfold the values at nuclear loci. The result is expected, because mitochondrial DNA is single copy, uniparentally transmitted, and non-recombining. Hence a sample size of *n* animals furnishes ¼ to ½ *n* genomes at mitochondrial than at genomic loci, depending on the effective sex ratio (Avise, 1994). The contrast between mitochondrial and nuclear diversities was particularly great in southern Africa, where allozyme diversities were higher than those in Kenya. Moreover, most of the mitochondrial diversity in southern Africa was in the Changara sample, and when Changara was not considered, the diversity estimate became even less. Indeed, the mitochondrial data suggest that *G. pallidipes* underwent a particularly stringent and prolonged bottleneck in southern Africa (Krafsur & Wohlford, 1999); this is not contradicted by the microsatellite data which might reasonably be expected to correlate with allozyme loci through linkage disequilibrium. The substantial allozyme heterozygosities in southern populations, however, offer no hint of an earlier bottleneck.

There is good historical evidence for an earlier bottleneck. A severe rinderpest epizootic in Africa beginning in 1889 was estimated to have wiped out more than 90% of the game and domestic cattle in southern Africa by 1896 (Ford, 1971). The epizootic was also severe in East Africa but tsetse distribution and abundance were not, to my knowledge, as well monitored as in southern Africa. Small, relic populations of *morsitans* group tsetse survived where their vertebrate hosts survived. Recoveries were slow. Resident entomologists in southern Africa and Uganda were able to map tsetse refugia and plot the expansion of populations as vertebrate host populations increased. After its disappearance, *G. pallidipes* was not recorded in Zimbabwe until 1942 (Lovemore, 1958). In principle, it could have remained undetected, even though tsetse officers had been sampling since 1930 (Ford, 1971: p. 365), but an equally likely explanation is that the Zimbabwe–Zambia *G. pallidipes* belt was established by immigrants from Mozambique via the Zambezi valley. Mitochondrial diversities suggest that a larger relic population survived in Mozambique than in Zimbabwe (Krafsur & Wohlford, 1999). Of six haplotypes detected in southern African populations, only one was shared between the Mozambique and Zimbabwe samples and it was found in 97% of the Zimbabwean and 21% of the Mozambique flies. Figure 3B shows that Mozambique clustered with populations in south-eastern Kenya rather than the Zimbabwean, and the same result was obtained when samples from Zambia and Ethiopia were included (Krafsur & Wohlford, 1999). The close affinity of Mozambique and south-east Kenyan populations supports Rogers & Randolph's (1993) suggestion, based on an analysis of temperature records, that suitable habitats exists for *G. pallidipes* from Kenya at least through Tanzania. It is likely that the same coastal habitat exists through Mozambique. On the other hand, allele *F* at microsatellite locus *CAG133* was predominating in southern Africa but was not found in Kenyan tsetse flies. Sampling *G. pallidipes* in Tanzania and Mozambique is indicated, although this would not be easy or inexpensive to do.

The departures from random mating among populations seem to be contradicted by ecological data that show great vagility in *G. pallidipes* (Vale *et al.*, 1984; Williams *et al.*, 1992). In

principle, the contradiction could be resolved if tsetse populations were in disequilibrium between selection and drift as they expand geographically from their refugia of a century ago. The expansion, however, has been gradual and it seems unlikely that a high degree of disequilibrium would pertain over such a large area, as is represented by the populations studied. Moreover, Ewens–Watterson neutrality tests (Watterson, 1986; Cornuet & Luikart, 1996) indicated that populations in each region were in mutation–drift equilibrium (data not shown).

In summary, mitochondrial variation confirms that *G. pallidipes* populations in southern Africa experienced a great reduction in numbers, many mitochondrial haplotypes becoming lost through drift. Microsatellite diversity was also reduced. On the other hand, allozyme heterozygosities in the same populations showed no loss of diversity and are not indicative of earlier bottlenecks, suggesting that allozymes are not selectively neutral. *F*-statistics based on allozyme loci in *G. pallidipes* indicated less genetic differentiation than estimates based on mitochondrial or microsatellite loci. This evidence is consistent with the operation of balancing selection that maintained allozyme diversities through a great reduction in population size. The adaptive significance of genetically based enzyme polymorphisms has been debated since Lewontin (1974) framed the question in modern terms. Black *et al.* (2001) have reviewed balancing selection in the context of insect genomics. Empirical and biochemical evidence in butterflies, *Drosophila*, sea anemones (reviewed by Kreitman & Akashi, 1995; Eanes, 1999), American oysters (Avise, 1992; Karl & Avise, 1992) and a beetle (Dahloff & Rank, 2000) indicates a role for selection at some allozyme loci at least. It seems that the tsetse fly *G. pallidipes* provides us with another example of balancing selection.

Experimental procedures

Sampling

Epsilon F3 cloth traps were used to collect *G. pallidipes*. The traps were baited with acetone, phenol and octenol (Torr *et al.*, 1989). The flies were killed, frozen in liquid nitrogen and shipped to Ames.

Sampling locations in Kenya were Marech (36°10′E, 3°49′S) in west central Kenya, Nguruman (36°05′E, 1°50′S) and Shompole *c.* 30 km away, in the south-west, Dakabuko (39°33′ E, 3°10′ S) and Alangoshira (39°38′E, 2°53′S) in east central Kenya, Shimba (39°03′E, 3°57′S) near the coast, and Kibwezi (38°27′E, 3°06′S) in the south-east.

The southern sampling locations included Mana Pools (29°21′E, 15°49′S), Makuti (29°21′E, 16°19′S), and Rekomitjie (29°21′E, 16°04′S), in Northern Zimbabwe; and Changara (33°08′ E, 16°19′S), west central Mozambique (Fig. 1). Mana Pools, Rekomitjie, and Makuti are about 30 km apart in a 60 km transect. Mana Pools and Rekomitjie are on the River Zambezi or its tributaries. In the dry season, game aggregate at the riverine sites. Makuti is on the escarpment at *c.* 620 m elevation. Changara was about 300 km east of Mana Pools and isolated from Zimbabwe by mountains and an area of dense human habitation that discourages *G. pallidipes*.

Population hierarchy

The eleven populations were apportioned into two regions, Kenya and southern Africa The hierarchical scheme is thus:

Kenya

East Rift Valley

Dakabuko

- Alangoshira
- Shimba Hills
- Kibwezi
- West Rift Valley
  - Nguruman
  - Shompole
  - Marech
- Southern Africa
  - Zimbabwe
    - Rekomitjie
    - Mana Pools
    - Makuti
  - Mozambique
    - Changara

Enzyme and DNA extractions

To extract enzymes, single tsetse were homogenized in 300 μl of grinding buffer containing sucrose, bromophenol blue, dithiothreotol, pH 8.6 tris-citrate buffer, and basic fuschsin. To extract DNA, a CTAB (hexadecyltrimethylammonium bromide) method was used. The protocol used for extracting DNA from allozyme homogenates was that of Williams *et al.* (1995).

Demonstration of single strand conformational polymorphisms

The methods of Black & DuTeau (1996) were used to amplify, electrophorese on acrylamide gels and silver stain mitochondrial polymorphisms, as previously described (Krafsur & Wohlford, 1999). Variation at cytochrome c oxidase subunit II and the large ribosomal subunit 16S2 rRNA was scored. The primers were N1-J-12585 and LR-N-12866 for *16S2*, and C2-J-3279 and C2-N-3494 for *COII* (Simon *et al.*, 1994). PCR reactions consisted of 10× PCR buffer, 0.4 mM dNTP, 1.5 mM $MgCl_2$, 4 μg BSA, 0.25 μM each of forward and reverse primers, template DNA, and *Taq* DNA polymerase for a final volume of 10 μl. Amplifications were performed in a PTC-100 thermal cycler (MJ Research, Waltham, MA).

Demonstration of microsatellite variation

Three microsatellite loci were evaluated, *GpCAG133* (GenBank accession no. AY033512), *GmcCA16C* (AF372513), and *GmsCAG2* (AF372514) (Baker & Krafsur, 2001). 15 μl PCR reactions consisted of 1.5 mM MgCl, 1.5 μl 10× polymerase buffer, 0.4 mM dNTPs, 0.5 μM HEX, FAM, or TET labelled forward primer, 0.5 μM reverse primer, 0.4 units Biolase® polymerase, *c.* 100 ng template DNA, and sdd water. The amplification profile was a 3-min initial denaturation at 94 °C, followed by thirty-four cycles of 4 s at 94 °C, 40 s annealing at 52 °C, and a 30 s extension at 72 °C. The final extension was 4 min at 72 °C. A master mix comprising 2.4 μl formamide, 0.5 μl blue dextran, and 0.6 μl GeneScan-350 [TAMRA] size standard (PE Biosystems, Foster City, CA) was added to 1.5 μl of each sample, and 1.3 μl of the final mixture was electrophoresed on a 4.5% acrylamide gel on an Applied Biosystems (ABI) Prism 377 DNA sequencer. The gel was analysed, and project and sample files were generated using ABI

GeneScan™ ver. 3.1.2. software. Allele sizes were scored by using ABI Genotyper™ ver. 2.5 software.

Enzyme electrophoresis

The allozymes included aconotate hydrolase, formaldehyde dehydrogenase, hydroxyacid dehydrogenase, peptidase, phosphoglucoisomerase, phospho-glucomutase, 6-phosphoglucose dehydrogenase, and triose phosphate isomerase. Gels consisted of 6.18% acrylamide plus 0.325% bis-acrylamide, 0.05% ammonium persulphate, and 0.15% TEMED in gel buffer. Buffer systems, electrophoretic methods, and staining procedures for tsetse have been previously described (Krafsur & Griffiths, 1997).

Genetic statistics

Nei's (1987) prescriptions were used to compute gene diversities and variances. An unbiased estimate of gene diversity at a nuclear locus is measured by the statistic $h_e = 2n(1 - \Sigma p_i^2)/(2n - 1)$, where p_i is each putative allele at the locus and n is the sample size. For mitochondrial diversity, the correction factor is $n/(n - 1)$. Gene diversity H_E for s nuclear loci is $\Sigma(h_e)/s$, with variance $\Sigma(h_e - H_E)^2/[s(s - 1)]$. H_E and h_e are the expected heterozygosities, on a scale of 0 −1.0, when mating is random and other Hardy–Weinberg assumptions apply. For mitochondrial haplotypes, h_e is the probability that a randomly chosen pair of organisms have different haplotypes.

For genomic loci, Wright's *F* statistics estimate departures from random mating at specified levels of population hierarchy. Gene frequencies were treated to analyses of variance by the method of Wright (1978). The Biosys-2 program (available from: <lamar.colostate.edu>, in the directory pub/wcb4) was used to do the computations.

Pair-wise Cavalli-Sforza and Edwards (see Wright, 1978) chord genetic distances were calculated by using Biosys-2. The unweighted pair group method by arithmetic averaging was used for the cluster analysis of the sampled populations. The Mantel test (Sokal & Rohlf, 1995: p. 813) was used to test the null hypothesis of no association between pair-wise population genetic distances measured at genomic and mitochondrial loci. The computations were carried out by using the Fortran program Mantel 1 (available from W.C. Black IV, Microbiology, Colorado State University). The original allozyme and mitochondrial gene frequencies have already been published (Krafsur *et al.*, 1997; Krafsur & Wohlford, 1999) and are not duplicated here.

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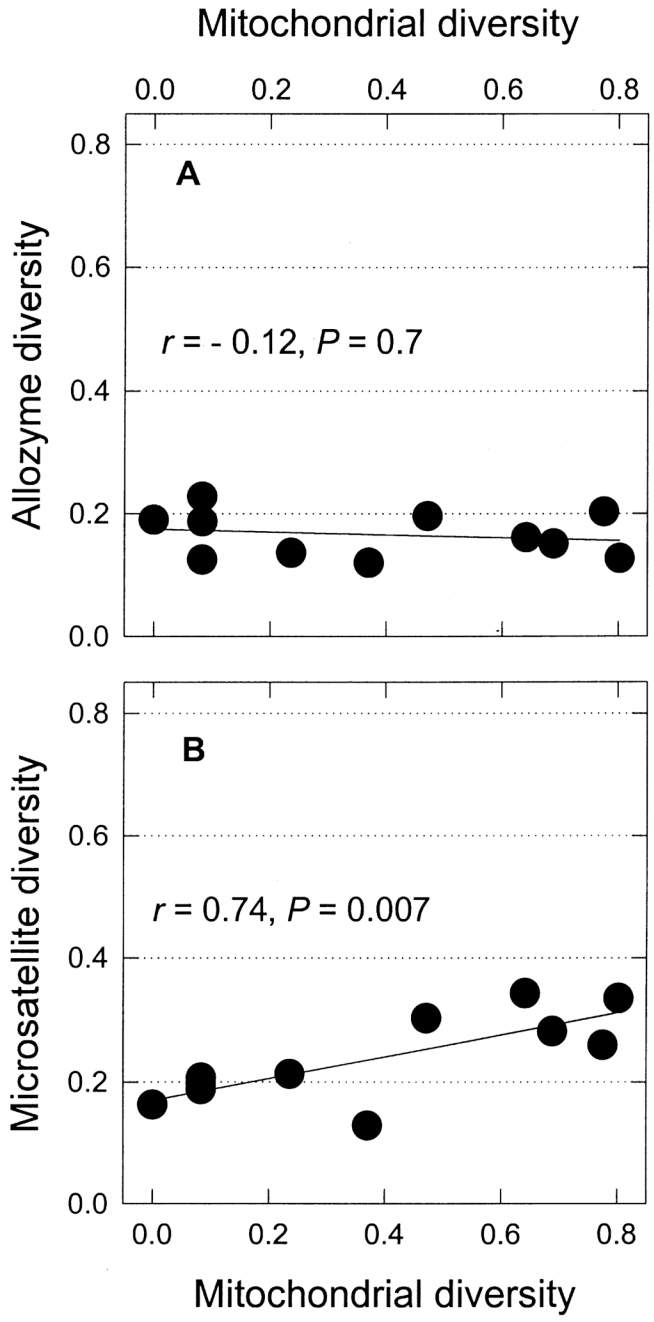

Figure 1.
Mean allozyme (A) and microsatellite (B) gene diversities plotted on the corresponding haplotype diversities.

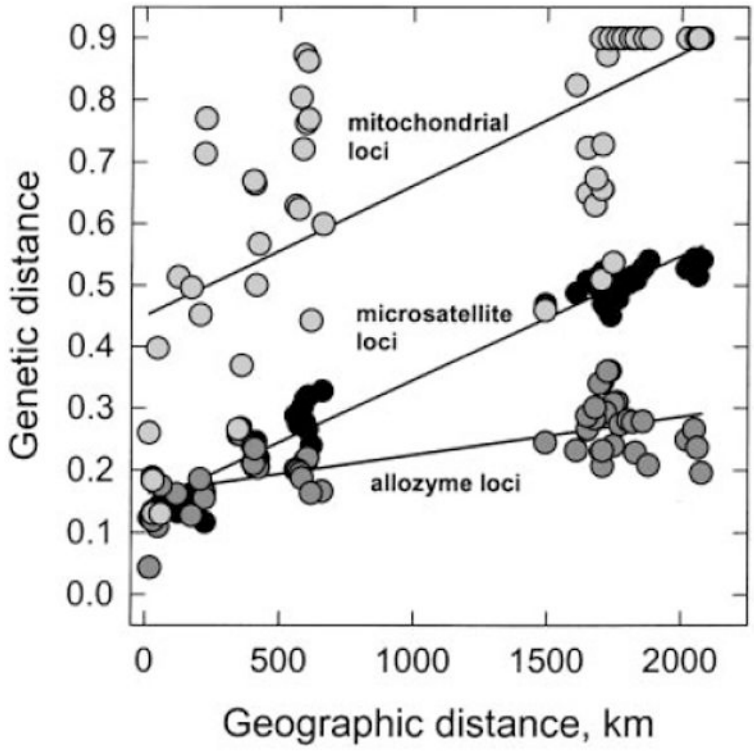

Figure 2.
Pair-wise Cavalli-Sforza & Edwards chord genetic distances regressed on corresponding geographic distances. The slopes are: for allozyme loci, 6.14×10^{-5}; for microsatellite loci, 2.02×10^{-4}; for mitochondrial loci, 2.12×10^{-4}.

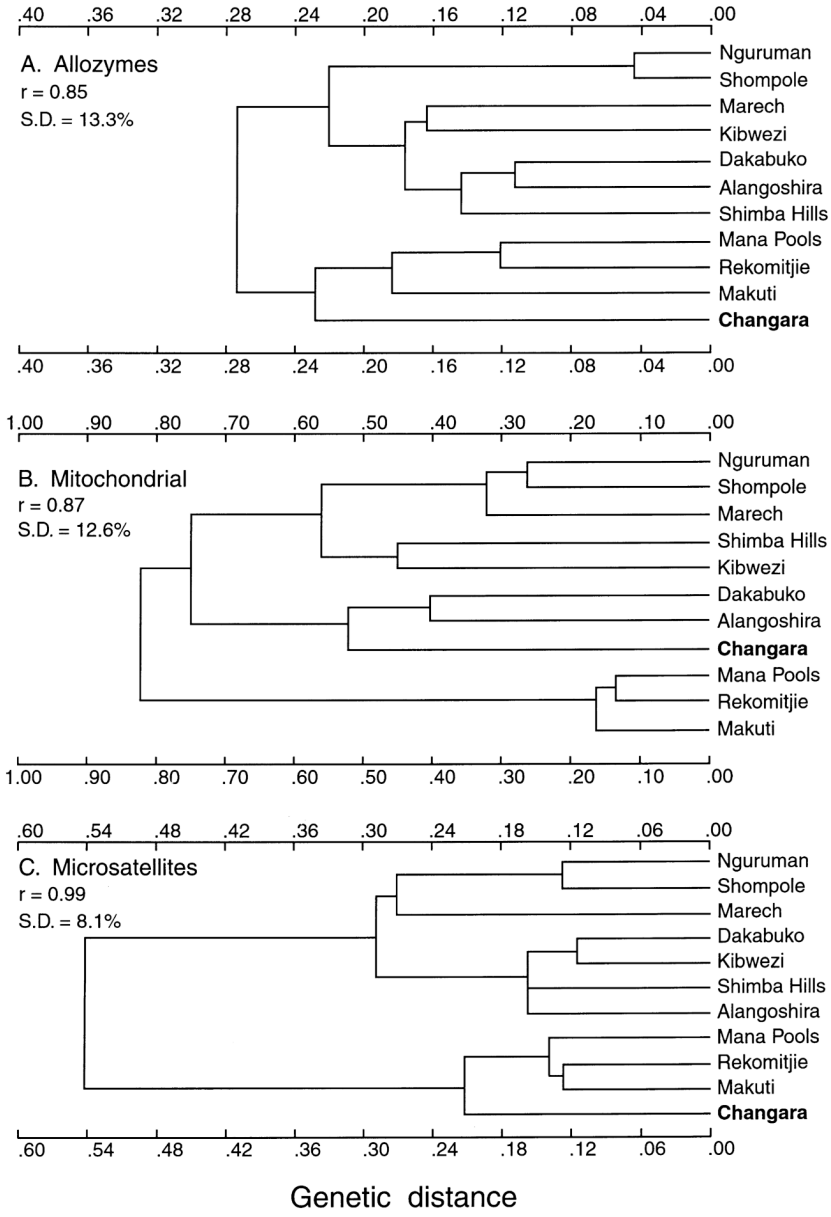

Figure 3.

(A) UPGMA dendrogram of Cavalli-Sforza & Edwards chord genetic distances for allozyme loci. (B) The same measure for mitochondrial haplotype genetic distances. (C) The same for microsatellite loci. Note that the genetic distance scales differ.

Table 1

Heterozygosities and *F*-statistics at microsatellite loci in eleven *G. pallidipes* populations

Locus	Heterozygosity	F_{IS}	F_{ST}	F_{IT}
GpCAG133	0.765	0.042	0.385	0.411
GmcCA16C	0.124	0.104	0.056	0.154
GmsCAG2	0.107	−0.065	0.027	–0.036
Mean	0.332	0.036	0.308	0.334

Table 2

Genomic and mitochondrial mean gene diversities (± SD) in *G. pallidipes* populations

Population	*N*	Allozymes	*N*	Mitochondria	*N*	Microsatellites
Marech	26	0.120 ± 0.086	24	0.370 ± 0.117	24	0.129 ± 0.064
Nguruman	49	0.125 ± 0.082	24	0.083 ± 0.075	24	0.188 ± 0.188
Shompole	49	0.136 ± 0.089	24	0.236 ± 0.109	24	0.213 ± 0.191
Kibwezi	26	0.203 ± 0.086	24	0.775 ± 0.062	24	0.260 ± 0.181
Alangoshira	49	0.161 ± 0.049	24	0.641 ± 0.099	20	0.343 ± 0.129
Dakabuko	30	0.151 ± 0.060	24	0.688 ± 0.093	24	0.282 ± 0.157
Shimba Hills	20	0.127 ± 0.069	48	0.802 ± 0.030	24	0.335 ± 0.169
Changara	47	0.199 ± 0.088	24	0.471 ± 0.105	24	0.303 ± 0.133
Rekomitjie	41	0.228 ± 0.068	24	0.083 ± 0.075	24	0.198 ± 0.118
Mana Pools	52	0.190 ± 0.083	24	0	24	0.163 ± 0.102
Makuti	24	0.187 ± 0.071	24	0.083 ± 0.075	24	0.207 ± 0.076

Table 3

Gene diversities averaged over loci in regionally grouped *G. pallidipes* populations

Group	No. of populations	No. haplotypes	No. alleles		Avg. gene diversities ± SD		
			Allozymes	microsats	mtDNA*	Allozyme†	Microsatellite‡
Kenya	7	13	29	14	0.504 ± 0.279	0.146 ± 0.029	0.250 ± 0.078
Southern Africa	4	6	24	13	0.156 ± 0.207	0.201 ± 0.019	0.218 ± 0.060
(less Mozambique)	3	3	23	10	0.055 ± 0.047	0.202 ± 0.023	0.189 ± 0.023

*Kenya $n = 191$, Southern Africa $n = 96$. Student's $t = 10.81$, $P < 0.001$.

†Kenya $n = 249$, Southern Africa $n = 157$. $t = 21.08$, $P < 0.001$.

‡Kenya $n = 164$, Southern Africa $n = 96$. $t = 34.9$, $P < 0.001$.

Table 4

Nested analysis of variance of gene frequencies in eleven *G. pallidipes* populations and Wright's fixation indices *Fxy*

Comparison, *x* – *y*	**d.f.**	**Variance component**	**(%)**	***Fxy***	**Estimated equiv. no. migrants (*Nm*)**
Allozyme loci					
Region* – total	1	0.10054	(34.2)	0.062	3.8
Populations – region	9	0.19372	(65.8)	0.127	1.7
Populations – total	803	0.29426		0.181	1.3
Microsatellite loci					
Region* – total	1	0.19531	(31.0)	0.197	1.0
Populations – region	9	0.08768	(69.0)	0.110	2.6
Populations – total	511	0.28299		0.285	0.6
Mitochondrial haplotypes					
Region* – total	1	0.26396	(34.2)	0.173	2.4
Populations – region	9	0.50852	(65.8)	0.403	0.7
Populations – total	277	0.77247		0.506	0.5

* Regions are Kenya and southern Africa.

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Appendix

Microsatellite allele frequencies in G. pallidipes

	Marech	Nguruman	Kibwezi	Shompole	S. Hills	Alango	Dakabuko	M. Pools	Rekomit	Makuti	Changara
Locus *GpCAG133*											
N	24	24	24	23	23	20	22	24	24	24	24
A	0.000	0.000	0.063	0.068	0.075	0.065	0.063	0.042	0.000	0.021	0.063
B	0.604	0.587	0.896	0.295	0.275	0.283	0.396	0.000	0.000	0.000	0.021
C	0.271	0.239	0.000	0.045	0.025	0.065	0.063	0.083	0.208	0.188	0.167
D	0.083	0.130	0.042	0.568	0.575	0.543	0.479	0.000	0.000	0.000	0.000
E	0.042	0.000	0.000	0.023	0.050	0.043	0.000	0.083	0.063	0.000	0.042
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.792	0.729	0.792	0.708
Locus *GmcCA16C*											
N	24	24	24	23	24	19	24	24	24	24	24
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.021	0.042
B	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.021	0.021	0.125
C	1.000	0.978	1.000	0.938	0.895	0.813	0.979	0.979	0.979	0.958	0.771
D	0.000	0.022	0.000	0.021	0.000	0.083	0.021	0.000	0.000	0.000	0.063
E	0.000	0.000	0.000	0.021	0.105	0.063	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Locus *GmsCAG2*											
N	24	24	24	24	24	19	23	24	24	24	24
A	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.042	0.042	0.083	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.021	0.000
C	1.000	1.000	0.896	0.935	0.868	0.979	0.938	0.958	0.938	0.896	0.979
D	0.000	0.000	0.104	0.043	0.132	0.021	0.063	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021

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