Preliminary study of wing morphometry in relation to tsetse population genetics

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COMPARATIVE MORPHOMETRIC ANALYSIS of shape variation in the wings of different tsetse species reveals close accordance with the phylogenetics of these species indicated by DNA sequence analysis. In practice, the morphometric analysis is economical and simple to carry out, suggesting that this could become a useful surrogate or complementary tool for large-scale studies of tsetse population genetics, designed to identify discrete population targets amenable to local elimination.

Introduction

Control of tsetse (Diptera, Glossinidae: vectors of African trypanosomiasis) can be achieved through a variety of techniques, including traps, insecticide-impregnated targets, live-baits, sequential aerial spraying (SAT), and sterile male release (SIT) (see Hargrove[1] for review). In most cases, however, the tsetse populations then tend to recover – either due to flies surviving the initial interventions, or immigration of flies from untreated regions, or both. To achieve and sustain local elimination of a target fly population, it is therefore preferable to define the area of intervention to include an entire panmictic fly population, such that natural immigration from neighbouring localities is of low likelihood. This is most readily achieved for isolated island populations, as shown by the elimination of *Glossina pallidipes* from the Island of Principe in 1914,[2] the eradication of *G. pallidipes* and *G. m. morsitans* from Antelope Island, Lake Kariba, Zimbabwe, in 1984,[1,3] and the elimination of *G. austeni* from Unguja Island of Zanzibar in 1997.[4] But for most mainland populations of tsetse, the geographical limits of target tsetse populations are less easily definable.

Application of population genetics techniques can reveal the existing level of population differentiation in tsetse, providing guidance on the distribution of genetically defined sub-populations. In essence, the population genetics models are used to estimate rates of gene flow between populations, which are taken as a surrogate for the rate of migration of individuals. Allozyme studies, for example, have revealed high levels of genetic differentiation within populations of *G. pallidipes* and other species of the morsitans group in East Africa,[5] suggesting that these species exist as a series of relatively isolated populations, each of which might be targeted separately for control interventions. Similarly, mitochondrial and microsatellite DNA analyses also reveal a high level of population structuring within species of the morsitans group in southern and eastern Africa,[6,7] and within some of the *G. palpalis gambiense* populations in West Africa.[8,9]

Extensive further studies of population structuring in tsetse seem appropriate as a guide to planning progressive control interventions, as envisaged by the AU-PATTEC initiative.[10] In addition, such studies could help in post-control monitoring for analysing the likely source of survivors or immigrants into treated areas – as shown for Triatominae, vectors of American trypanosomiasis.[11] To minimize the use of expensive techniques of DNA-sequence analysis for such studies, we present here a preliminary comparison of geometric wing morphometry as an inexpensive surrogate for genetic analysis. This work was carried out at the level of tsetse species and species-groups, as a prelude to further studies of within-species differentiation.

Materials and methods

The insects. Samples were received as individuals or groups of flies in 70% ethanol, from colonies maintained at the FAO/IAEA laboratories in Seibersdorf, Austria, and from the CIRDES laboratories at Bobo Dioulasso, Burkina Faso. Additional samples of *G. p. gambiense* were collected by trapping along the Kou valley, Burkina Faso (Table 1).

Wing morphometry. Wings were removed and dry-mounted between two microscope slides. The right wing of each specimen was photographed using a digital camera. Images of each wing were subsequently digitized and 7 cartesian coordinates (homologous landmarks defined by vein intersections; Fig. 1) were recorded automatically using TPSdig software (version 1.39).[12] The *x, y* coordinates were subjected to generalized procrustes analysis (GPA)[13] and subsequently to a thin-plate spline analysis[14] using TPSrelw software (version 1.35)[15] and TPS regr (version 1.26),[16] allowing visualization of shape differences as deformation grids. The analysis produces variables subdivided into uniform and non-uniform components of shape changes. To offset the problem of small sample sizes, a principal component analysis of the shape variables delivers fewer shape components ('relative warps'), explaining most of the shape variance within the data set. The relative warps were subsequently analysed by discriminant analysis. Size differences were assessed using centroid size (CS), an isometric estimator of size derived from the GPA superimposition procedure. Finally, mean Mahalanobis distances were used in a cluster analysis to construct a UPGMA dendrogram (Unweighted Pair Group Method with Arithmetic Mean). Multivariate analyses and graphs were completed using JMP® version 4.0.5 (SAS Institute Inc. 2001) and Intercooled STATA 8.2 for Windows (Stata Corporation 2003)

DNA sequence comparison: For comparison with the wing morphometry, we used available partial sequences of the ribosomal DNA internal transcribed spacer-2 (ITS2) downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) (Table 1). These sequences were aligned using Clustal-X and analysed by Neighbor-

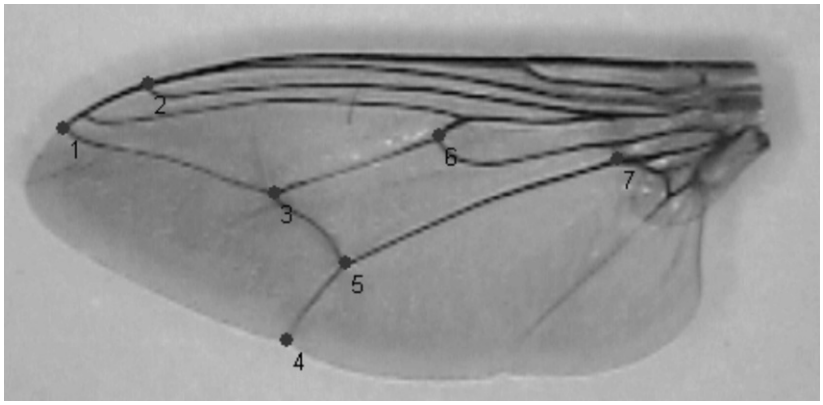

Fig. 1. Slide-mounted tsetse wing showing landmarks used for morphometric analysis.

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Table 1. *Glossina* specimens used in the study.

Species group	Species	Source of material for morphometry (*n*)	ITS2 GenBank accession nos
morsitans	*G. morsitans s.l.*	FAO/IAEA, CIRDES (21)	AF021360; AF021359; F021358
	G. pallidipes	FAO/IAEA (5)	AF021357(1); AF021356(2)
	G. swynnertoni	FAO/IAEA (4)	AF021355
palpalis	*G. palpalis gambiensis*	FAO/IAEA, CIRDES, and Kou valley (16)	AF024505
	G. fuscipes	FAO/IAEA (4)	AF021352
	G. tachinoides	CIRDES (4)	AF021353
fusca	*G. brevipalpis*	FAO/IAEA (3)	AF022361(1); AF022360(2)

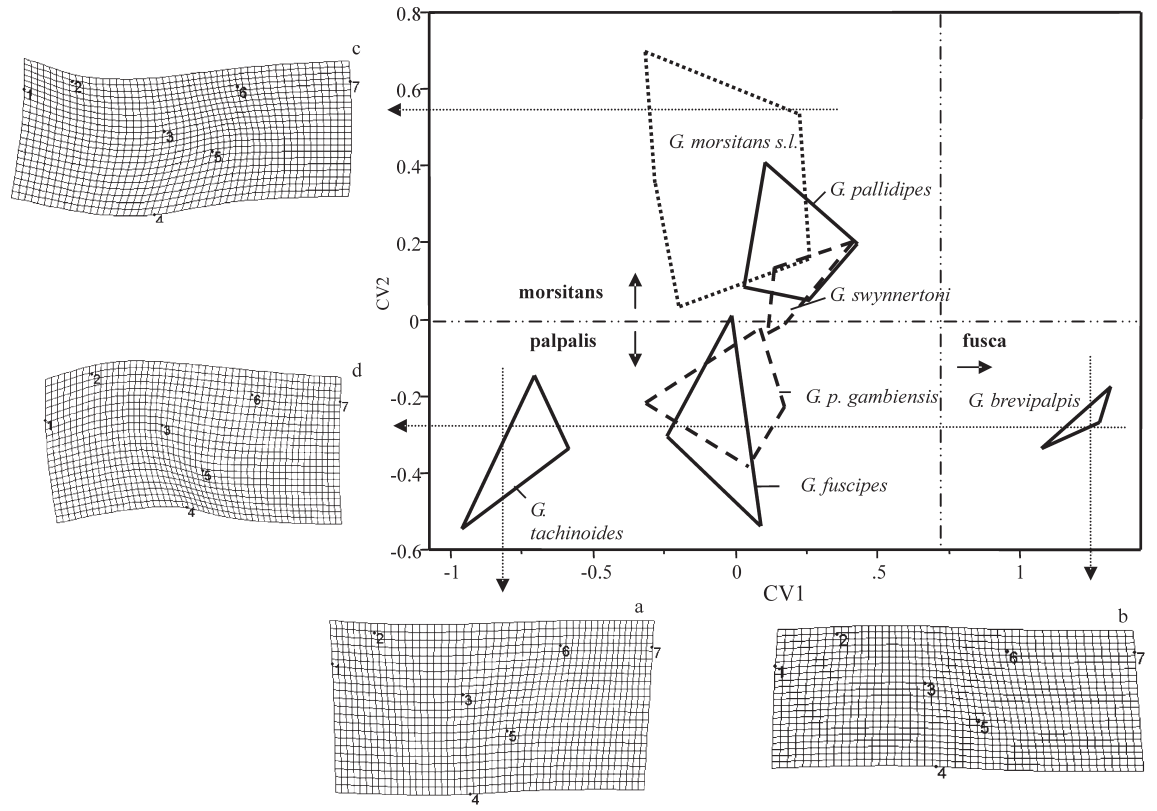

Fig. 2. Discriminant analysis of the morphometric data, showing the distribution of specimens in the space defined by the first two canonical variates (CV1 and CV2). **a–d** are thin-plate splines showing, by deformations from the mean, shape differences of the wings that correspond to the indicated species/species groups on both axes (landmarks on the deformation grids are numbered as in Fig. 1).

Joining using the Kimura-2-parameter model of base substitution to construct phylogenetic trees with 1000 bootstrap replicates.

Results

The first five relative warps (shape components) accounted for 90.6% of the variance in the total data set, and were used as input for the discriminant analysis. Regressing centroid size against the first relative warp gave no significant correlation ($r = 0.002$), suggesting that size is not the primary factor influencing the major shape differences. Figure 2 shows the discrimination of the three main species groups by wing morphometry. The discriminant model gave correct reclassification scores of 100% for pooled members of the fusca and morsitans groups and 95.2% for palpalis (4.2% assigned to morsitans group). These reclassification scores were 'almost perfect' (kappa = 0.96).[17] The first two canonical vectors (CV) together accounted for 84% of the total heterogeneity (CV1–56% and CV2–28%). The thin-plate spline representations (Fig. 2a,b) show that most of the shape change is associated with a relative elongation of the wing, and this separates fusca from the morsitans and palpalis species groups. Figure 2c,d show that the secondary factor of shape change is related to the relative arrangement of vein junctions, and clearly discriminates between the morsitans and palpalis groups.

Analysis of the ITS2 sequences revealed three major clades, with good bootstrap support. These correspond to the three species groups, and show clear congruence with a cluster analysis of the morphometric data (Fig. 3).

Discussion

The 31 currently recognized species and subspecies of *Glossina* are customarily placed into three species groups which are sometimes given subgeneric status[18] – the fusca group (subgenus *Austenina*), palpalis group (subgenus *Nemorhina*), and morsitans group (subgenus *Glossina*). These groupings are based primarily on morphological features of the adult genitalia,[19] although they also reflect differences in distribution, habitat and behaviour.[20] Species of the fusca group typically occur in lowland rain forests of West and Central Africa (exceptions being *G. longipennis* and *G. brevipalpis* in drier regions of eastern Africa); species of the palpalis group are more usually associated with riverine vegetation, but also extend into savanna regions between river systems; while species of the morsitans group are primarily associated with drier savannas. In this study, the geometric analysis of wing morphometry successfully recovered not only the species, but also the three species-groups.

As initially shown by Solano *et al.*[8] for *G. p. gambiense*, a degree of correlation can

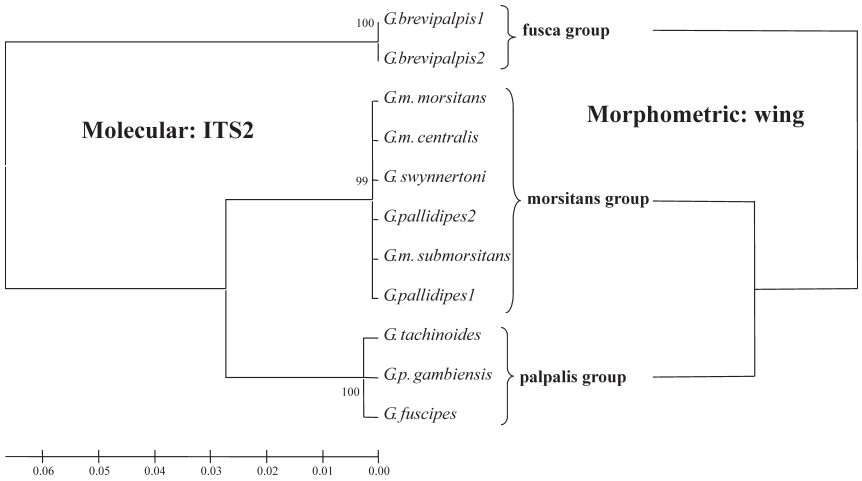

Fig. 3. Comparison of Neighbor-Joining k2p linearized tree (left) with UPGMA cluster analysis of Mahalanobis distances from wing morphometrics (right). Numbers indicate per cent bootstrap support from 1000 replicate analyses.

be found between estimates of population structuring based on analysis of microsatellite DNA, and comparative wing morphometrics. Using linear wing morphometrics, these authors showed clear separation between *G. p. gambiense* populations of Senegal and Burkina Faso, but not between populations within Burkina Faso which were revealed by comparative analysis of microsatellite DNA sequences. Genetic separation of the Senegal populations from those of Mali was also confirmed by Marquez *et al*.[9] using comparisons of mitochondrial DNA sequences. In our study, the very high congruence over seven species and subspecies between genetic comparisons based on a ribosomal DNA sequence, and phenetic comparisons based on geometric morphometry, suggests that the geometric analysis is a more sensitive surrogate for the DNA sequence comparisons. It appears, moreover, that wing shape may represent a relatively neutral trait that is not heavily modulated by ecological adaptation or environmental constraints.

In practice, data collection for geometric analysis of wing shape is relatively simple. The wings can be dry mounted between microscope slides and then either scanned (using a computer scanner) or photographed with a digital camera. The resulting image can then be processed using freely available software (e.g. http://life.bio.sunysb.edu/morph/), or sent as an e-mail attachment to a reference laboratory for further analysis. Such a procedure is simpler and much less costly than current techniques for DNA extraction and sequencing, and offers opportunities for rapid processing of large samples of field-collected material covering the entire distributional range of target species and subspecies of tsetse. This would permit detailed analysis of population structuring to identify the geographical limits of discrete or panmictic populations, which would represent targets for control interventions that would be least likely to suffer from post-control reinvasion. In addition, as shown for Triatominae,[11] such studies can also be used for post-intervention monitoring, providing a way to confirm whether any newly encountered tsetse are survivors from the initial control interventions (indicating a local control failure) or are immigrants from a neighbouring population of that species (perhaps indicating a breakdown of control barriers).

In the context of the African Union initiative to eliminate the problem of tsetse and trypanosomiasis (AU-PATTEC),[10] we believe that these techniques could be particularly applicable for defining geographical areas amenable to large-scale elimination of the tsetse populations. There is a wealth of evidence that tsetse control is feasible, but also that it is difficult to sustain over the long term.[1,21,22] By contrast, local elimination of tsetse is sustainable, but generally held to be feasible only for geographically constrained situations such as islands.[1,2,4] The task of population genetics studies is, in a sense, to find and define those biogeographical 'islands' of tsetse distribution on mainland Africa, and our study shows that this may be feasible on a large scale using the techniques of geometric morphometry, with confirmation from smaller-scale studies using the more expensive methods of DNA sequence comparisons.

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