sites for the monitoring programme. In practical terms, populations with a uniform distribution may be less complicated to suppress than a population aggregated into discrete foci (e.g. the Niayes infestation (orchards) in Senegal).

Calculating apparent density of *G. fuscipes* or other riverine flies based on a grid square value can be misleading as they are assumed to be relatively restricted to riverine vegetation cover.

Basic data on spatial distribution of tsetse in the survey area and their apparent densities determined at different seasons will enable the seasonal movements of tsetse to be assessed with respect to altitude and vegetation/climatic characteristics. These data will enable rates of dispersal throughout the area to be assessed. Analysis of the structure of the population in terms of its age and sex ratios will also contribute to planning of control/eradication.

## 3.3. GENETIC ANALYSIS

### 3.3.1. Objectives of Genetic Analysis in the Context of AW-IPM Programmes

One purpose of genetic analysis of tsetse flies may be to determine the degree of genetic isolation of neighbouring populations. This will contribute to determining whether or not a population is really isolated from another or whether there is gene flow, indicating movement of individuals between those tsetse populations. This is obviously important when considering AW–IPM, as re-invasion of cleared areas has to be prevented.

Despite the relatively low ability of tsetse flies to disperse, in comparison with other dipteran pests such as screwworm flies, there is a high potential for fly re-invasion into areas where control operations have been undertaken. Especially with *palpalis* group tsetse flies, which are considered to be quite restricted to riverine vegetation, it is often difficult to determine the extent to which they can disperse from one area of suitable habitat (river system) to another. An indirect method of determining the likelihood of this is through genetic characterization. Subpopulations, or demes, that exchange flies will be genetically much more homogenous (same gene frequencies) than those between which there is little or no genetic exchange (different gene frequencies). PCR techniques can now be used to rapidly characterize tsetse populations genetically, using either mitochondrial or microsatellite DNA markers (Solano et al. 1999, 2000, Krafsur 2003, Marquez et al. 2004). It is therefore desirable to collect samples for this purpose from the target area being surveyed and from neighbouring areas of tsetse infestation. Results of such analyses will contribute to confirming or otherwise, the assumed degree of isolation of the target population. Obviously this should be carried out early in the planning stage.

Recent studies using remote-sensing technologies have shown that in areas subject to human encroachment (in East and West Africa) tsetse fly populations become fragmented and in some cases isolated. Finding these "biological islands" by assessing their genetic isolation will undoubtedly help to target these populations for sustained vector control, possibly even eradication. However, such populations need to be identified and characterized prior to control operations. Molecular and morphometric techniques seem to have the potential to rapidly identify the levels of epidemiologically important population substructuring in tsetse vectors.

### 3.3.2. Principles of Genetic Analysis

The null hypothesis is the Hardy-Weinberg rule stating that gene frequencies will be homogeneous among sampled populations if matings are random, the genetic variation selectively examined is neutral, the mutation rate is negligible, and the sampled populations are infinitely large. Most deviations from these initial assumptions are caused by departures from random mating within and among populations and population sizes that are not large. Thus, we can test hypotheses about gene flow and population sizes by sampling a series of populations and estimating the amounts and spatial components of gene diversity.

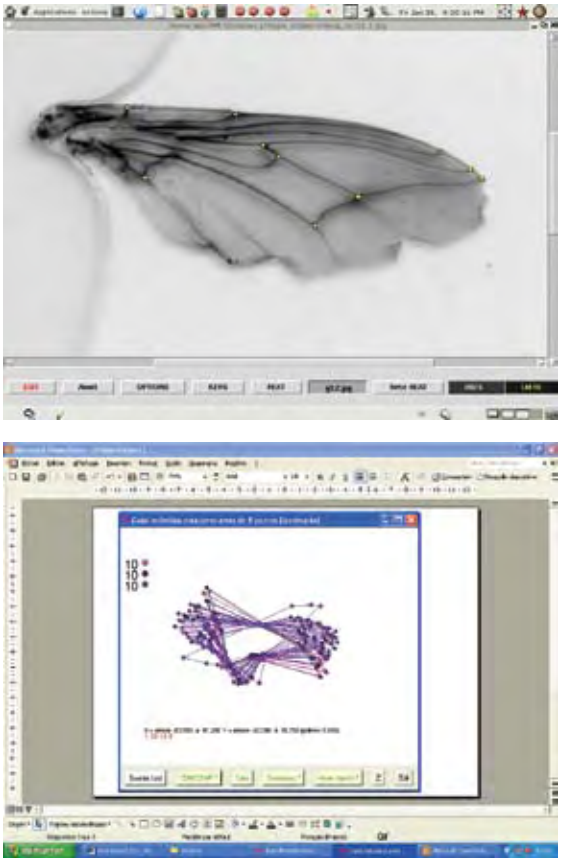
With free exchange of reproducing flies and random mating, gene frequencies in populations approach homogeneity, and among-population variance in gene frequencies is small. Variance in gene frequencies increases with greater genetic divergence. The standardized variance in gene frequencies among subpopulations is termed $F_{st}$. $F_{st}$ represents the departure from random mating *among* subpopulations. The departure from random mating *within* subpopulations is $F_{is}$ and this statistic is normally close to zero (Weir and Cockerham 1984). An $F_{is}$ estimate significantly greater than zero, when averaged over loci, can indicate that the sample contained individuals from two or more subpopulations that differ in their allelic frequencies — the Wahlund effect. $F_{st}$ can be related to migration and dispersal by various theoretical models. According to Wright's island model, the mean number of breeding migrants, $Nm$, in a generation is related to $F_{st}$, thus $F_{st} = (1 + 4Nm)^{-1}$. In principle, numerically little gene flow among populations prevents genetic differentiation by drift (Gooding and Krafsur 2005).

### 3.3.3. New Possibilities: Geometric Morphometrics of Tsetse

Morphometric characters are related to growth and development, and they are usually continuous. Traditionally, they were estimates of distances between anatomical points called landmarks ("Traditional morphometrics"). More recently they have come to be the coordinates of these landmarks in a given system of orthogonal axes ("Geometric morphometry") (Rohlf and Marcus 1993). The few examples of the use of geometric techniques in medical entomology are found in Phlebotominae (vectors of Leishmaniosis) and Triatominae (vectors of Chagas disease), and very recently this technique has been shown to be promising in tsetse flies (Patterson and Schofield 2005). These examples indicate that geometric morphometry makes it possible to distinguish species, subpopulations, ecotypes and even successive generations by the configuration of a few landmarks on the wings (**Figure 3.14, upper**). Besides its evident contribution to insect systematics, the main epidemiological contribution of morphometrics to medical entomology has been to help decision making in the development of vector control strategies: thus, it has been possible to recognize the geographic origin of invading or re-infesting specimens (Dujardin et al. 1997). The high discriminatory power of geometric morphometrics could be used here to delineate natural barriers between subpopulations within the main species.

A significant advantage of this technique compared to others is the simplicity of data acquisition and its low cost. Basic laboratory equipment needed consists of: a mono- or binocular microscope, a simple scanner, a computer. Specialized software is freely available at http://www.mpl.ird.fr/morphometrics. Raw data collected from the wing consists of a set of coordinates corresponding to a few homologous, landmarks. The main lines of the

FIGURE 3.14
**Example of geometric morphometrics of tsetse wings**



(upper) scanned wing with ten easily identifiable landmarks for geometric morphometrics; (lower) scanned wings (here, for instance, three samples of ten flies, each) have been transformed into polygons and are ready for shape analysis

*J.-P. DUJARDIN AND P. SOLANO, IRD*

analysis are the computation for each wing of one estimator of size, the "centroid size", and of a set of shape variables called "partial warps".

The "partial warps" are then used as input for any kind of multivariate analysis comparing groups, populations or species. Size may be studied separately by univariate analysis. The independence between size and shape is generally good, and may be measured by regression techniques. The possible differences among groups may be directly visualized by the technique of "thin-plate splines".

## 3.3.4. Collection of Samples for Genetic Analysis

### *3.3.4.1. Sample Size*

The analysis can be carried out on small numbers of flies — a target number of 30 flies (ten males and 20 females) from each species and from each area suspected to be geographically isolated are sufficient.

### *3.3.4.2. Preservation of Fly Samples*

The flies can be dried immediately after capture, to avoid them becoming contaminated with fungus or rotting, or can be preserved in alcohol (70% ethanol). Flies from different locations should be stored in separate labelled containers.

Ideally, when flies have been caught and given to the dissection team, processing would be recommended for each fly as follows:

- wings should be carefully removed with forceps and stored in separate, labelled, eppendorf tubes for subsequent morphometric analyses,
- for genetic analyses, using microsatellite DNA markers only two to three legs of each fly need to be taken and also put in numbered eppendorf tubes. For studies using mitochondrial DNA the whole tsetse individual is required,
- hence, each fly needs to have a unique identifier related to the identity of the trap, which will allow its genotyping and morphometric identity,
- before embarking on the collection of samples for genetic analysis, it is necessary to identify a laboratory at which the analyses can be carried out and make the necessary arrangements. There are currently no commercial laboratories providing that service and analyses generally are part of a research project. Among the potential laboratories that may have an interest, and currently have the capacity for genetic analysis are the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria, the Centre International de Recherche Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo Dioulasso, Burkina Faso, the Department of Entomology, Iowa State University, USA and the L'Institut de Recherche pour le Développement (IRD)/Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) (IRD/CIRAD) in Montpellier, France.

## 3.4. REPORTING

On completion of a survey the procedure and results have to be adequately reported, whether it be to a government ministry, donor(s), in a scientific journal, conference proceedings, elsewhere, or in a combination of those forms. This will provide a historical record of what was done, of the methodology that was used and of the results obtained that could then be used for planning future activities.

The report should follow a standard format, providing all the necessary information required for a person who was not involved in the survey to understand and interpret the data at some time in the future. The suggested layout is as follows:

**1. Introduction**

This should explain the problem that is being addressed, the reasons for undertaking the survey and its objectives.

**2. Materials and Methods:**

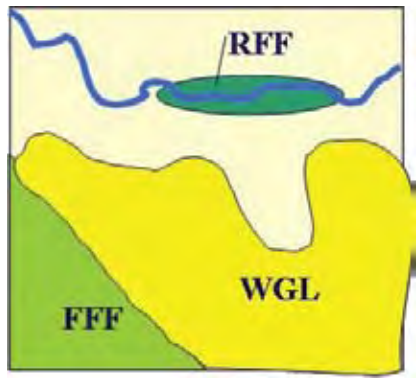
**Study area —** Describe the geographical location of the study area, how it was chosen and its descriptive characteristics with regard to climate, vegetation, geography, etc. Maps should be included here illustrating the location of the site in relation to the country/region. In addition to a basic map indicating the location, a land use or vegetation map of the area needs to be included (e.g. **Figure 3.15**) in order to describe the planning of the survey and to further describe the area. Reference will be made to any previous knowledge of tsetse distribution and abundance from the same location.

**Materials used —** Give details of the type and source of satellite images used and of how they were interpreted.

**Survey design —** This section will describe the number of sampling sites, the way in which they were selected, and their locations and other characteristics. The sampling method need to be described, along with the justification for selecting that method. Other information will include all the relevant description of the survey procedures such as when it was done (year and seasons), methodology of the sampling (how many times in a day, what time, what was done with the flies caught, what parameters were recorded for the flies and for other survey components, e.g. climate).

**Statistical analysis —** Commonly, data on fly density is transformed to normalize the data, i.e. to transform the data to a normal distribution, and logarithmic transformations are often chosen as the most appropriate. This section should describe the type of transformation, if any, carried out and the reasons for doing so. In epidemiological surveys in which data on disease parameters are collected, or where environmental

FIGURE 3.15
**Example of a sketch map showing vegetation types in a survey area**




RFF: riparian forest, WG: woody grassland, FFF: forest

parameters are measured (rainfall, vegetation type, etc.) statistical tests are likely to be carried out to explore relationships between these various parameters. These and any other statistical treatment of the data are described in this section.

**3. Results**

How many flies? What species? What was the apparent density? What was the spatial distribution? What was the seasonal effect? What relationships were detected with vegetation and climatic factors?, etc.

**4. Discussion**

What do the results signify? How do they compare with what was found previously/elsewhere? What lessons were learned from the survey methodology? What will be done next?

**5. Conclusions**

What is the outcome of the survey? For example, would a control or eradication programme be recommended or did the survey results suggest that this would not succeed or would not be feasible within the available budget. What next steps might need to be undertaken based on the outcome of the survey?

Ideally, maps of the survey results should be made available in the public domain (e.g. GeoNetwork (Cecchi and Mattioli 2007, Cecchi et al. 2008) under the auspices of FAO) in order to make available updated continental tsetse distribution maps.

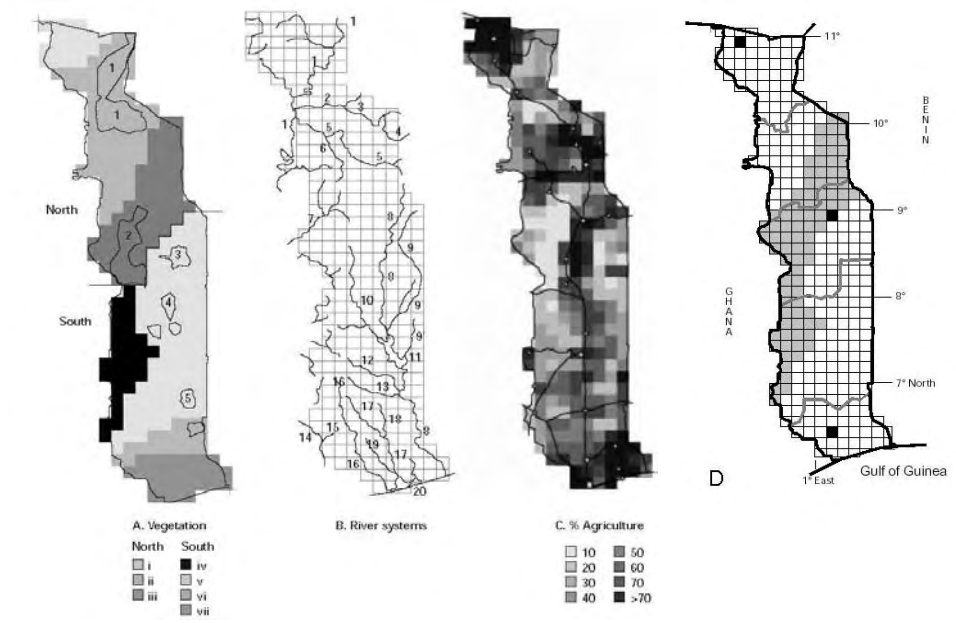
## 3.5. EXAMPLES OF GRID-BASED TSETSE SURVEYS

### 3.5.1. Togo

In Togo, to allow systematic country-wide surveys, the country was divided up into grid squares or sample units measuring 0.125° latitude/longitude. This raster, or grid base of 311 identical cells was used for the sampling frame. Different variables were sampled in every grid square (**Figure 3.16**) for a country-wide survey of tsetse flies and other related epidemiological parameters (Hendrickx et al. 1999).

The survey in Togo was conducted not with an immediate view to control or eradication but to produce country-wide distribution and abundance maps for ecological and epidemiological analysis that could be used for planning future activities. The survey made use of grid-based (raster) surveying and recording of data in an area infested with *G. tachinoides*, *G. p. palpalis*, *G. m. submorsitans*, *G. longipalpis* and *G. fusca fusca*. In order to obtain grid cell-specific fly density values, 1:200 000 maps were consulted to select survey sites representative in terms of the prevailing dominant vegetation types, and also taking into account the local drainage systems and the accessibility of the terrain. Locally, within each site, the field teams selected what they perceived to be suitable tsetse habitats according to expected fly species, and positioned clusters of a minimum of five and an average of 12 tsetse traps for 24–48 hours. Thus a total of 653 different survey sites were sampled in 305 of the 311 grid squares comprising 14 620 trapping days.

FIGURE 3.16
**Results from a grid-based survey in Togo**




(a) Vegetation gradient in northern Togo: (i) northern savannah (sandstone), (ii) Sudanian savannah, (iii) dry forest. Vegetation gradient in southern Togo: (iv) humid forest; (v) Guinean savannah, (vi) transition zone, and, (vii) protected areas (game and forest reserves), (b) the grid base with a GIS layer showing major rivers, (c) agricultural intensity expressed as the percentage of land under cultivation, including fallow land, and main roads and towns, and (d) a map of Togo with the grid squares superimposed with the Atakora mountain range, that divides the country, shown in grey-shaded grid squares

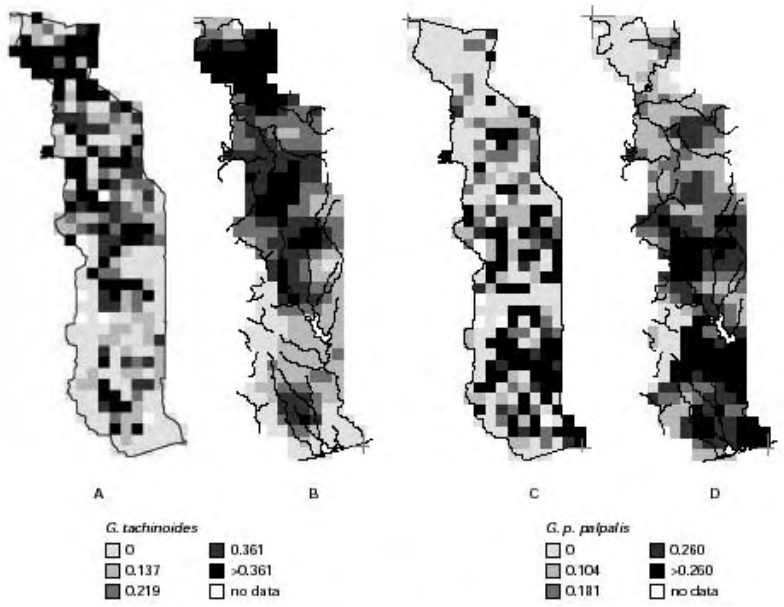
*Source:* Hendrickx et al. 1999



The tsetse survey took place in 305 grid squares with an average of 12 biconical traps per trapping site. On average 2.1 sites were sampled per grid square for 24 or 48 hours. As sampling took place at different seasons at different locations within the country, the data were processed to make seasonal adjustments and for spatial smoothing. Spatial smoothing was done to reduce the amount of random variation by using a GIS tool to average each grid value with that of the eight, or less, adjacent grids. Using the same technique it was possible to "estimate" the likely value for grid squares for which no data were obtained. Hendrickx et al. (1999) believed that after spatial smoothing, the prevalence maps produced revealed better and more accurate patterns than those produced directly from raw data. Efforts concentrated on the precise limits at the edges of the fly distributions (**Figure 3.17**).

In terms of logistics, the Togo survey was conducted by five different mobile teams operating simultaneously. Each team was assigned one out of five administrative regions of the country, roughly corresponding to a 1:200 000 map each. The country was divided into four major seasonal clusters from dry monomodal in the north to wet bimodal in the

FIGURE 3.17
**Abundance maps (log10 (fly + 1)) of riverine tsetse flies (subgenus Nemorhina) per grid**




Maps (a) (*Glossina tachinoides*) and (c) (*Glossina palpalis palpalis*) show raw data adapted for seasonal influences, maps (b) (*G. tachinoides)* and (c) (*G. palpalis palpalis*) show 3 × 3 spatial smoothed data.

*Source:* Hendrickx et al. 1999



south. Seasonal clusters were obtained after hierarchical clustering of a series of remotely-sensed and ground-measured ecoclimatical variables. The authors concluded that the methodology provided an ideal transect survey that was representative for most coastal West African countries.

### 3.5.2. The Southern Rift Valley, Ethiopia

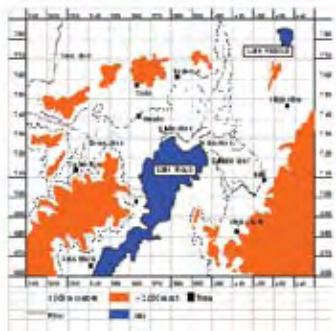
In the Southern Rift Valley of Ethiopia (**Figure 3.18**), an AW-IPM programme started in 1998 with the aim to create a tsetse-free zone. The programme was initiated with a baseline tsetse survey that had the objective of determining the feasibility of eradicating *G. pallidipes*, eventually from an area of 25 000 km$^2$ but starting with a block of approximately 10 000 km$^2$ (Vreysen et al. 1999).

The baseline survey was planned to define the limits of the tsetse distribution, to assess the apparent density of the tsetse populations, to confirm the degree of isolation and the number of species infesting the area. Within the block in which activities were to start (block 1), the 103 10 × 10 km grid squares (**Figure 3.19**) were divided amongst five field teams (**Figure 3.20**). Each team consisted of a team leader, two technicians and several assistants, and was equipped with transport, traps and full field equipment to make them fully mobile. The teams were trained in the selection of trap sites, deployment of traps

FIGURE 3.18
**Satellite image of the Lake Abaya region of the Ethiopian Southern Rift Valley**

NASA

FIGURE 3.19
**Map of the Southern Rift Valley survey area, Ethiopia, with 10 x 10 km grid overlay**

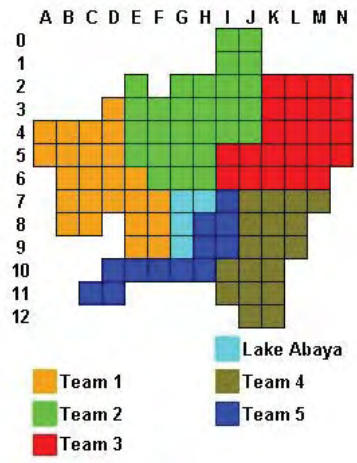


*Source:* Vreysen 2000

according to a standardized protocol, and fly identification and catch recording. In addition they were trained in the classification of vegetation into ten classes. The initial task for the teams was to familiarize themselves with the grids assigned to them. They studied each grid, recording the distribution of vegetation types, and checking on access routes, updating the survey maps to show new tracks.

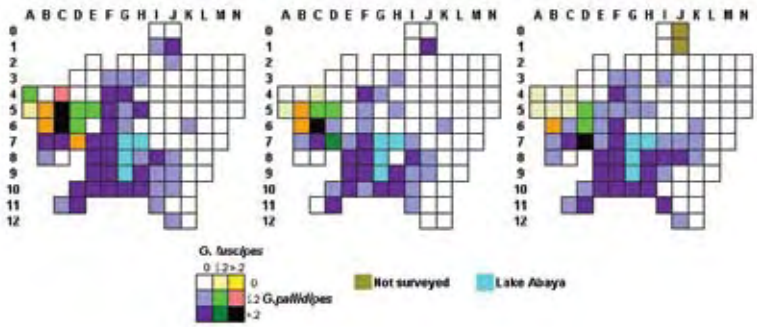
The initial survey plan called for the deployment of at least 20 traps in each grid square for 72 hours in a three-month cycle for one complete year to observe seasonal changes. The traps were deployed in each of the available vegetation classes, and over the full range of altitudes up to more than 2000 metres above sea level (masl). The maximum altitude of trapping was adjusted by experience to ensure that the maximum trapping altitude was well above the tsetse distribution limit. The actual number of traps deployed in the grids at

FIGURE 3.20
**Division of the Southern Rift Valley (Ethiopia) survey area into blocks and survey teams based on a 10 x 10 km grid**




*Source:* Vreysen 2000

FIGURE 3.21
**Results (apparent density of *Glossina fuscipes* fuscipes and *Glossina pallidipes*) of the survey in the Southern Rift Valley (Ethiopia)**




(left) January–March 1999; (middle) April–June 1999; (right) July–September 1999

the margin of the project area varied depending on the proportion of the grid inside the project boundary.

After the first cycle of trapping the trap sites were fixed, and all trap locations identified by global positioning system (GPS) readings. Trap altitude was ascertained by digital altimeter, referred to the nearest map primary or secondary datum point (Ethiopia Department of Surveys maps 1:50 000 series).

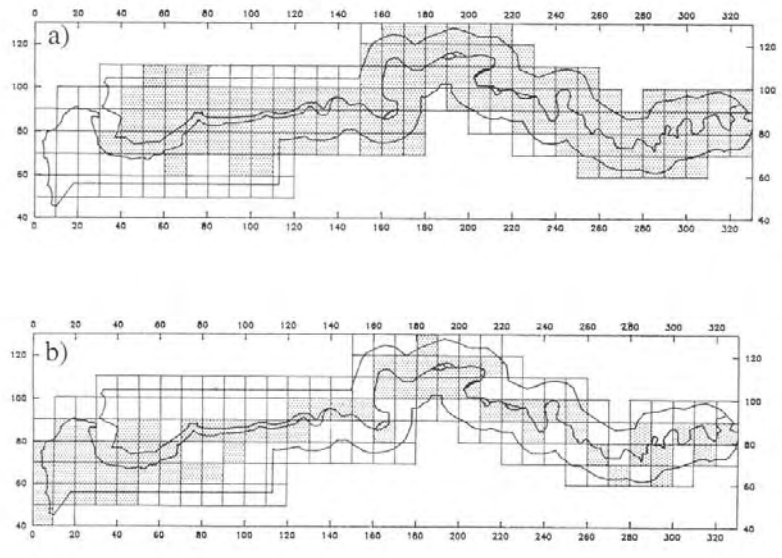
Only a quarter of the grid squares caught tsetse on each of four surveys carried out during the year. This indicates the importance of surveying over more than one period. A single baseline survey would not have given an accurate impression of the tsetse distribution (**Figure 3.21**).

### 3.5.3. The Gambia

A third example of a large-scale, country-wide survey was the survey of The Gambia, conducted by the International Trypanotolerance Centre (ITC) and the Gambian Department of Livestock Services (DLS) in the 1980s (Rawlings et al. 1993). Although this survey was conducted without the benefits of GPS and GIS software, there are lessons to be learned from the approach taken. A grid structure was used for the country, based on 10 × 10 km UTM grid squares found on standard 1:50 000 scale maps of the country. Trapping was conducted at 1654 sites over an area of 10 000 km$^2$, using odour-baited F3 box traps deployed in each grid square for 24 hours at each site. The survey was conducted by two four-man mobile field teams who covered 80% of the area in six months. This survey did not target all areas equally, but was directed at the densest habitats that were considered most likely to be favourable to tsetse (woodlands) in each 10 × 10 km square. The suitable sites to deploy traps were identified using 1:50 000-scale aerial photographs taken in 1982 in combination with the national survey maps and finally, field verification of their suitability. Repeat surveys were carried out in grid squares from which no tsetse were initially

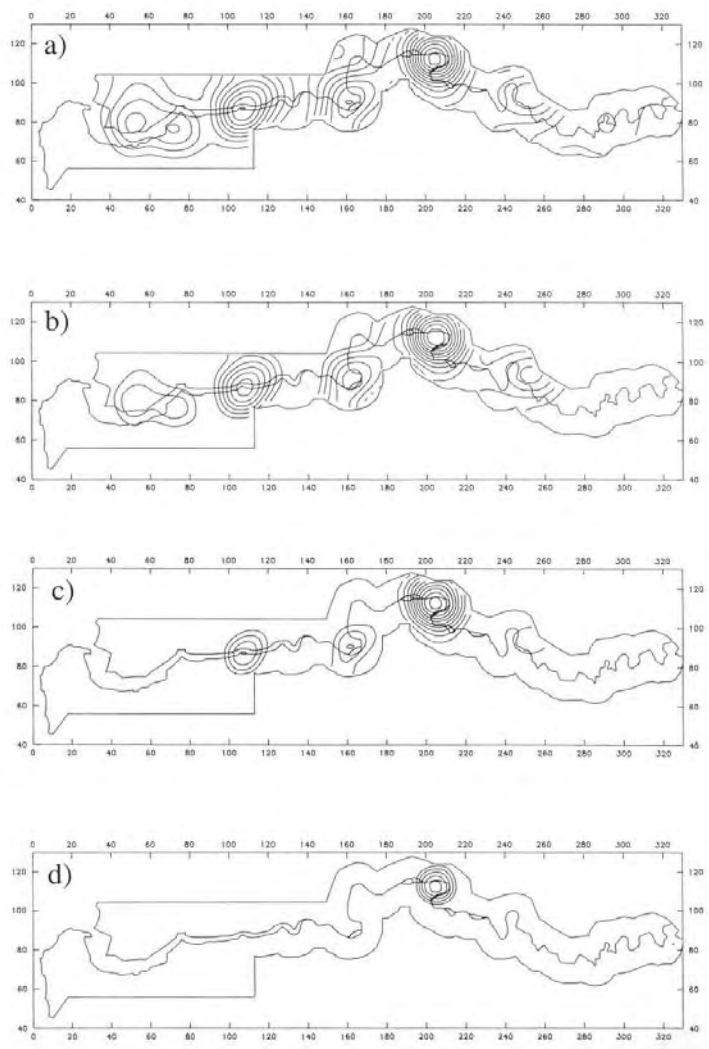
FIGURE 3.22

**The distribution of (a) *Glossina morsitans submorsitans*, and (b) *Glossina palpalis gambiensis* in The Gambia showing the 10 × 10 km UTM grid squares in which each species was distributed**




*Source:* Rawlings et al. 1993

FIGURE 3.23
**Smoothed contours with the mean annual abundance of *Glossina morsitans submorsitans* in The Gambia based on the 1989-1990 country-wide survey**




(a) areas with two or more flies/trap/day (FTD), (b) areas with FTD of five or more, (c) areas with FTD of ten or more, (d) areas with FTD of 20 or more

*Source:* Rawlings et al. 1993



caught. Map coordinates were taken from the same, hard copy 1:50 000 scale maps. It was subsequently possible to convert those map references into digitized UTM or decimal degrees coordinates in order to produce GIS maps of the distribution of the two tsetse species, *G. m. submorsitans* and *G. p. gambiensis,* in the country (**Figure 3.22 and 3.23**).

**Additional information collected:** Habitat type; sightings of warthogs, or warthog activity (preferred host of *G. m. submorsitans* in The Gambia). The data were stored in dbase3 files and mapped using SURFER software.

### 3.5.4. The Ghibe Valley, Ethiopia

This example is included in order to show how a smaller, more detailed, 1-km$^2$ grid square based survey was carried out in the Ghibe valley of Ethiopia. The survey protocol that was implemented used three odour-baited biconical traps per square kilometre, and therefore quite intensive data was acquired over a relatively small area. Due to the intensive nature of the survey, carried out by a single small team, the area of the survey could not be covered over the short period of a single period. As seasonal variations in apparent density are well known to occur, it was therefore necessary to attempt to make a seasonal adjustment for data obtained at different times of the year. This was done using data obtained from a small number of traps in a representative area for long-term monitoring over a period of 10 years prior to the survey, using a similar technique to that used in the Togo survey (see 3.5.1.).

An additional feature of the Ghibe survey was that it was complemented by other georeferenced data on households, cattle crushes, and cattle grazing areas (the latter was also done in The Gambia, leading to better epidemiological knowledge determining disease risk estimates). The Ghibe survey differed from the timing that would be expected for surveys in that it was carried out in the middle, rather than at the beginning of a small-scale (450 km$^2$) control operation using insecticide-treated cattle, to enable a better epidemiological understanding of the events taking place rather than to plan future events. Some spatial analyses (cross-tabulations and regression analyses) of tsetse distribution and cattle grazing areas were carried out.

## 3.6. EQUIPMENT AND MATERIALS REQUIRED

### 3.6.1. Materials/equipment

The equipment necessary for the trap assistants is minimal:

- some traps and screens (about 10% of the total distributed) to replace those that may be destroyed or stolen;
- some monitoring traps and their accessories;
- books and pencils to note all observations and the results of monitoring (see monitoring);
- material for the monitoring: containers, alcohol for preserving samples, etc., and
- accessories such as a machete, hammer, string, etc.

**Traps** — Depending upon the design of trap decided upon, they may be obtained by an existing commercial supplier, e.g. Vestergaard-Frandsen, contracted to commercial tailors/manufacturers who are provided with a design, and preferably a sample, or by local tailors contracted to make the traps under project supervision. As referred to earlier, it is preferable to have the traps made by a commercial supplier in order to ensure a standard quality, although this may be more expensive. Furthermore, if local tailors/manufacturers are used

it is advisable to provide them with suitable cloth from a good supplier in order to ensure that it meets the required specifications.

**Odour attractants** — Deciding upon which odour attractants to use depends upon the species of tsetse present and which attractants are available that are effective for those species (**Tables 3.2** to **3.5 and** 3.1.4) sufficient number of standard dispensers dispensing at suitable and approximately known standard rates will be needed; some of these, for example for cow urine or acetone do not have very precise specifications and can usually be obtained cheaply locally. If synthetic phenol-based attractants are to be used these can be either obtained ready prepared, or put into sachets locally. If they are to be processed locally a roll of polythene tubing of a suitable thickness will be required and a machine for heat-sealing the sachet. It may be sufficient to use simple attractants, such as acetone and cow urine that may both be obtained locally, although purchasing large quantities of acetone can be difficult due to its flammability and restrictions on its purchase related to drug processing. It is therefore advisable to source all these items a sufficient time in advance. Cow urine functions best when it is at least three weeks old and should therefore be collected and stored before it is needed.

**Satellite images —** Cost and availability depend upon the resolution required, but they are becoming more readily available and affordable: United States Geological Survey (USGS), National Aeronautics and Space Administration (NASA), the FAO, and the European Union.

- **LandSat TM:** https://zulu.ssc.nasa.gov/mrsid/ From the US Government NASA LandSat 5, LandSat 7 satellites;
- **Quickbird:** http://digitalglobe.com/;
- **SPOT vegetation satellite:** http://www.vgt.vito.be From the European satellite image processing and archiving centre, Belgium, providing free access to the vegetation image catalogue of data from the SPOT 4 and SPOT 5 satellites.
- **National Oceanic and Atmospheric Administration (NOAA) Advanced Very High Resolution Radiometer (AVHRR)** meteorological satellite data: http://www.gis.ssd.nesdis.noaa.gov/

**Global positioning system (GPS) instruments —** It is important to determine what coordinate system is to be used to georeference data and set all GPS instruments to the same system, using the same datum that is appropriate for the location of the survey. Coordinate data can/should be stored in the instruments and downloaded using the appropriate software to avoid unnecessary data entry that is often accompanied by data entry errors and the consequent need for time-consuming data-entry verification. There are a limited number of manufacturers of GPS instruments (Garmin, Magellan, Trimble) and links to those manufacturers are given in Annex 4.

**Vehicles —** Appropriate numbers of suitable (4WD) vehicles capable of transporting traps, people and smelly attractants; motorbikes – may sometimes be more suitable for

checking traps, although not necessarily for trap deployment, in rough terrain or in wet conditions.

**Computers —** Sufficient capacity and speed to handle large data sets and memory and speed consuming software for GIS. Most modern computers now meet the necessary specifications.

**Software —** Standard software: e.g. Microsoft Word®; Access®; Excel®, plus specialised GIS software, e.g. IDRISI or ArcView® / ArcGIS®. ArcView® or its replacement, ArcGIS®, is the standard software used for GIS whilst Microsoft Access® is widely used for data storage, analysis and reporting and is compatible with the GIS software.

A high-resolution colour printer, capable of printing A3 size is required for printing maps.