Acta Tropica xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Population studies of *Glossina pallidipes* in Ethiopia: emphasis on cuticular hydrocarbons and wing morphometric analysis

M.N. Getahun[a,b,*], G. Cecchi[c], E. Seyoum[a]

[a] Addis Ababa University, Department of Zoological Sciences, P.O. Box 1176, Addis Ababa, Ethiopia
[b] Max Planck Institute for Chemical Ecology, Department of Neuroethology, Jena, Germany
[c] Food and Agriculture Organization of the United Nations, Sub-Regional Office for Eastern Africa, CMC Road, P.O. Box 5536, Addis Ababa, Ethiopia

ARTICLE INFO

Article history:
Available online xxx

Dr. Emiru Seyoum passed away on January 1st 2014 we dedicate this article to his memory.

Keywords:
Glossina pallidipes populations
Cuticular hydrocarbons
Wing morphometrics
GIS
Ethiopia
SIT

ABSTRACT

Tsetse flies, like many insects, use pheromones for inter- and intra-specific communication. Several of their pheromones are cuticular hydrocarbons (CHCs) that are perceived by contact at close range. We hypothesized that for a successful implementation of the Sterile Insect Technique (SIT), along with proper identification of target area and target species, the target tsetse populations and the sterile flies must chemically communicate with each other. To study the population structuring of *Glossina pallidipes* in Ethiopia, CHCs were extracted and analyzed from three tsetse belts. As a comparative approach, wing morphometric analysis was performed. The analysis of the relative abundance of CHCs revealed that populations of *G. pallidipes* from the Rift Valley tsetse belt showed a distinct clustering compared to populations from the other two belts. The spatial pattern of CHC differences was complemented by the wing morphometric analysis. Our data suggest that CHCs of known biological and ecological role, when combined with wing morphometric data, will provide an alternative means for the study of population structuring of *Glossina* populations. This could aid the planning of area wide control strategies using SIT, which is dependent on sexual competence.

1. Introduction

Tsetse flies are the only biological vectors of *Trypanosoma vivax, T. congolense*, etc., the causative agents of African animal trypanosomosis (AAT or nagana) and *T. brucei gambiense and T. brucei rhodensience* causative agents of human trypanosomiasis, which continue to cause poverty and threaten human health in Sub-Saharan Africa (Simarro et al., 2012; WHO, 2013). The burden of trypanosomosis persists despite the massive and diverse control efforts undertaken over decades (Vreysen, 2006). Intervention approaches have included destructive methods such as bush clearing (Potts and Jackson, 1952; Ford et al., 1970), elimination of wild tsetse hosts (Potts and Jackson, 1952), intensive application of persistent insecticides (Davies and Blasdale, 1960; Putt et al., 1980), as well as ecologically friendly approaches such as traps and targets (Vale et al., 1986). The Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) placed emphasis on the sustainability of tsetse eradication and advocated the adoption of an area-wide approach, whereby the elimination effort is directed against the entire vector population within a circumscribed area (Vreysen et al., 2000; Kabayo, 2002). To achieve this goal, the intervention campaign should integrate a combination of the various tools, among which are trapping, pour-on insecticides, insecticide treated targets, the Sequential Aerosol Technique (SAT) and the Sterile Insect Technique (SIT) (Vreysen et al., 2000; Adam et al., 2012). SIT depends on the use of laboratory-reared sterilized males that are released into the field in high numbers. For the technique to be effective, the laboratory strain should be fit enough to compete with wild counterparts in dispersal, survival, mobility, as well as in sexual competence (Itô and Yamamura, 2005; Vreysen et al., 2013). Insects including hematophagous species such as tsetse flies are highly dependent on olfaction besides other cues such as vision for host detection and finding mating partners (Gibson and Brady, 1988; Bursell, 1990; Green, 1993; Takken and Knols, 1999; Gikonyo et al., 2003; Carlson et al., 2005; Gurba et al., 2012). One of the challenges to successful SIT implementation can be the phenotypic variation between tsetse populations, even within the same country. Genetic variation can result from a patchy distribution and

* Corresponding author at: Max Planck Institute for Chemical Ecology Department of Neuroethology, Jena, Germany. Tel.: +49 3641 571456; fax: +49 3641 571402.
E-mail addresses: mgetahun@ice.mpg.de, merid.negash@yahoo.com (M.N. Getahun).

http://dx.doi.org/10.1016/j.actatropica.2014.04.015
0001-706X/

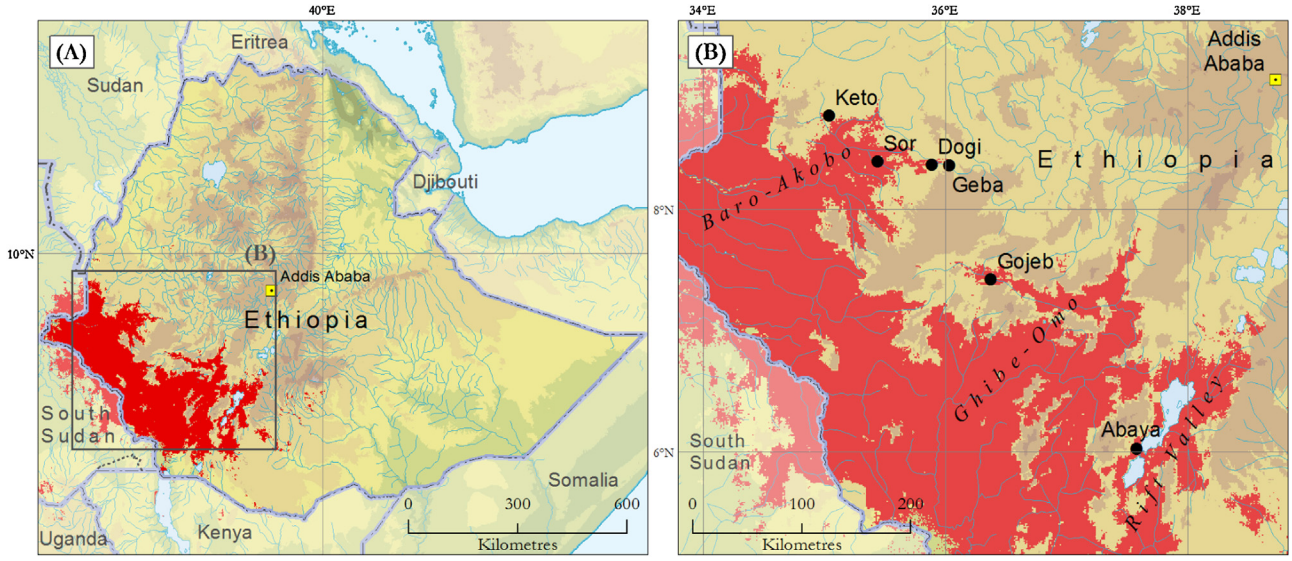

Fig. 1. (A) Study zone in Ethiopia. (B) Tsetse sampling sites: Keto, Sor, Geba and Dogi from Baro-Akobo tsetse belt; Gojeb from Ghibe-Omo tsetse belt; and Abaya from Rift Valley tsetse belt. *G. pallidipes* predicted distribution in Ethiopia (in red) was derived from the Programme Against African Trypanosomosis Information System (www.fao.org/ag/paat-is.html).

from geographic isolation (Krafsur and Griffiths, 1997; Solano et al., 1999; Wohlford et al., 1999; Krafsur, 2003; Itô and Yamamura, 2005). Genetic variation between tsetse populations might affect mate recognition and acceptance (Krafsur, 2003), and this may pose particular risks when a laboratory colony established from one population is used to target different populations in a country or region during a SIT campaign.

Population differentiation studies that focus on bio-chemical molecules associated with mate-finding behavior can provide valuable information for successful planning and implementation of SIT campaigns. In tsetse flies, as in many Dipterans, most known sex pheromones are cuticular hydrocarbons (CHCs) (Langley et al., 1975; Carlson et al., 1978, 1984, 2005), that provide both inter- and intra-specific information (Howard and Blomquist, 2005). We hypothesize that the analysis of CHC profiles of tsetse populations can give insights into population structuring, which could be related to sexual behavior and sexual competence. Indeed, as CHCs are species-specific either in type or in quantity, they are increasingly used to delimit species and play an important role in chemotaxonomy (Kather and Martin, 2012). In addition to CHCs, wing morphometric analysis is extensively used for population studies, as it has the capability to detect minimal morphological variations between cryptic species, which are not detected by classical morphological approaches (Matias et al., 2001; Dujardin et al., 2009; Kitthawee and Dujardin, 2009). For tsetse flies, as well as for other insects, it has been shown that the results of morphometric analysis are often comparable to those obtained through more conventional approaches such as genetic-marker based phylogenetic classification (Patterson and Schofield, 2005; Camara et al., 2006; Solano et al., 2010; Lee and Lin, 2012; Kaba et al., 2012).

The objective of this investigation was to characterize populations of *Glossina pallidipes* of three tsetse belts in Ethiopia using CHCs analysis. Wing morphometric analysis was utilized as a comparative approach. The environments of these three regions were also characterized using Geographic Information Systems (GIS) and a selection of geospatial datasets, which are increasingly utilized for planning and implementation of interventions against trypanosomosis using SIT (Cox and Vreysen, 2005; Cecchi and Mattioli, 2009; Simarro et al., 2010; Cecchi et al., 2014). Results are discussed in the light of their potential application in SIT planning.

2. Materials and methods

2.1. Description of study sites

In Ethiopia, four tsetse belts can be distinguished: Rift Valley, Ghibe-Omo, Baro-Akobo and Blue Nile the last three named after major rivers. The Ethiopian Rift Valley, which is part of the great East African Rift Valley, is where most of Ethiopia's lakes are located. The Ghibe-Omo is a tributary of the Omo River, which flows southwards along the Rift Valley into Lake Turkana at the Ethiopian/Kenyan border. Baro-Akobo is a river basin in the south-western part of the country with different tributaries that defines part of Ethiopia's border with South Sudan. The Blue Nile is also a vast tsetse belt of the river basin bordering the Blue Nile State of Sudan. *G. pallidipes* is one of the major tsetse species in Ethiopia in terms of distribution and economic importance, and it is widely distributed in all but the Blue Nile tsetse belt (Ovazza, 1956; McConnell et al., 1970; Langridge, 1976; Fuller, 1978; Leak et al., 1993a,b). Cattle and humans are preferred hosts, as shown by studies from the Ghibe-Omo tsetse belt (Leak et al., 1993a,b).

In this study, tsetse flies were sampled from selected sites of the three tsetse belts where *G. pallidipes* is found. The sites include Abaya (Rift Valley belt), Gojeb River (Ghibe-Omo belt), Sor, Dogi, Geba and Keto rivers (Baro-Akobo belt) (Fig. 1). In addition to field sites mentioned above, the *G. pallidipes* population from the laboratory in Kality was also studied. Kality is located near Addis Ababa, and the *G. pallidipes* strain reared in Kality was established by collecting flies from the Rift Valley tsetse belt. All tsetse flies used in the present study were collected in 2008.

2.2. Trapping and identifying tsetse flies

NG2G traps (Brightwell et al., 1991) made from 100% polyester textile (Permanet Vestergaard-Frandsen) were used. Phenol + cow urine was used as attractant (Vale et al., 1988), and traps were spaced 250 m apart. All collection sites were geo-referenced using Garmin 12 Channel GPS (USA). Traps were emptied every 24 h. The details on the trapping procedure can be found in previous reports (Merid Negash et al., 2007).

The following morphological taxonomic characters were used to identify the trapped flies as *G. pallidipes*: last two tarsal segments

Please cite this article in press as: Getahun, M.N., et al., Population studies of *Glossina pallidipes* in Ethiopia: emphasis on cuticular hydrocarbons and wing morphometric analysis. Acta Trop. (2014), http://dx.doi.org/10.1016/j.actatropica.2014.04.015