Infection, Genetics and Evolution xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Genetic comparison of *Glossina tachinoides* populations in three river basins of the Upper West Region of Ghana and implications for tsetse control

Y. Adam [a], J. Bouyer [b,c,*], G.-K. Dayo [d], C.I. Mahama [a], M.J.B. Vreysen [e], G. Cecchi [f], A.M.M. Abd-Alla [e], P. Solano [g], S. Ravel [h], T. de Meeûs [g]

[a] *Veterinary Services Department of MOFA, P.O. Box 97, Pong-Tamale, Ghana*
[b] *Cirad, UMR INRA-CIRAD Contrôle des maladies animales exotiques et émergentes, F-34398 Montpellier, France*
[c] *Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Elevage et de Recherches Vétérinaires, BP 2057, Dakar – Hann, Senegal*
[d] *Centre International de Recherche-Développement Sur l'Elevage en Zone Subhumide (CIRDES), 01 BP 454, Bobo-Dioulasso, Burkina Faso*
[e] *Insect Pest Control Laboratory, Joint FAO/IAEA Program of Nuclear Techniques in Food and Agriculture, P.O. Box 100, Wagramerstrasse 4, A 1400 Vienna, Austria*
[f] *Food and Agriculture Organization of the United Nations, Sub-regional Office for Eastern Africa, CMC Road, P.O. Box 5536, Addis Ababa, Ethiopia*
[g] *IRD, UMR 177 IRD-CIRAD Intertryp, CIRDES 01, BP 454, Bobo-Dioulasso 01, Burkina Faso*
[h] *IRD, UMR 177 IRD-CIRAD Intertryp, TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*

ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 17 March 2014
Accepted 24 March 2014
Available online xxxx

Keywords:
Tsetse
Population genetics
Vector control
Population structure
Dispersal

ABSTRACT

Tsetse flies are the cyclical vectors of African animal trypanosomosis (AAT) and human African trypanosomosis (HAT). In March 2010, the Government of Ghana initiated a large scale integrated tsetse eradication campaign in the Upper West Region (UWR) (≈18,000 km^2) under the umbrella of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC).

We investigated the structuring of *Glossina tachinoides* populations within and between the three main river basins of the target area in the UWR. Out of a total sample of 884 flies, a sub-sample of 266 was genotyped at nine microsatellite loci. The significance of the different hierarchical levels was tested using Yang's parameters estimated with Weir and Cockerham's method. A significant effect of traps within groups (pooling traps no more than 3 km distant from each other), of groups within river basins and of river basins within the whole target area was observed. Isolation by distance between traps was highly significant. A local density of 0.48–0.61 flies/m^2 was estimated and a dispersal distance that approximated 11 m per generation [CI 9, 17]. No significant sex-biased dispersal was detected.

Dispersal distances of *G. tachinoides* in the UWR were relatively low, possibly as a result of the fragmentation of the habitat and the seasonality of the Kulpawn and Sissili rivers. Moreover, very high fly population densities were observed in the sample sites, which potentially reduces dispersal at constant habitat saturation, because the probability that migrants can established is reduced (density dependent dispersal). However, the observed spatial dispersal was deemed sufficient for a *G. tachinoides*-cleared area to be reinvaded from neighboring populations in adjacent river basins. These data corroborate results from other population genetics studies in West Africa, which indicate that *G. tachinoides* populations from different river basins cannot be considered isolated.

1. Introduction

Tsetse flies are the cyclical vectors of African animal trypanosomosis (AAT) – nagana – and of human African trypanosomosis (HAT) – sleeping sickness. HAT is only reported sporadically from Ghana, the last case having been diagnosed in the year 2000 (Simarro et al., 2010). As a result, the risk of infection in the country is presently considered marginal (Simarro et al., 2012). By contrast, AAT is a major constraint to the development of more efficient and sustainable livestock production systems in Ghana, and the disease prevents utilization of abundant natural pastures across the country.

Increased parasite resistance to the commonly used trypanocidal drugs were observed in Ghana, due to their widespread and indiscriminate use by livestock keepers (Turkson, 1993). Over the

* Corresponding author. Address: ISRA-LNERV, BP 2057, Dakar – Hann, Senegal. Tel.: +221 771843070; fax: +221 33 832 36 79.
E-mail addresses: yahadamco@yahoo.com (Y. Adam), bouyer@cirad.fr (J. Bouyer), charlesdayo@yahoo.fr (G.-K. Dayo), cimahama@yahoo.com (C.I. Mahama), M.Vreysen@iaea.org (M.J.B. Vreysen), Giuliano.Cecchi@fao.org (G. Cecchi), A.M.M.Abd-Alla@iaea.org (A.M.M. Abd-Alla), philippe.solano@ird.fr (P. Solano), sophie.ravel@ird.fr (S. Ravel), thierry.demeeus@ird.fr (T. de Meeûs).

http://dx.doi.org/10.1016/j.meegid.2014.03.023
1567-1348/

years, the Veterinary Services Directorate of the Ministry of Food and Agriculture has thus been committed to controlling AAT. Despite past control efforts, AAT remains prevalent in Ghana, particularly in the Upper West Region (UWR) (Adam et al., 2012).

In 2001, the African Union launched the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), advocating the eradication of tsetse populations as the most sustainable way of controlling trypanosomosis in sub-Sahara Africa. Under the umbrella of the PATTEC, Ghana is collaborating with Burkina Faso in a sub-regional initiative aimed at creating tsetse-free areas across their common border.

The UWR shares its northern and western border with Burkina Faso and it was selected as a target zone for the first phase of the PATTEC initiative in Ghana. In March 2010, the Government of Ghana initiated a large-scale integrated campaign for the eradication of tsetse, with the ultimate goal of removing riverine tsetse populations from ≈18,000 km^2 (Adam et al., 2013). The project integrated several tools such as the sequential aerosol technique (SAT), insecticide-treated targets, insecticide-treated cattle and ground spraying. As a result of the inclusion of a SAT-component, it was estimated that the applied strategy reduced the target tsetse populations by 98% within one month of the start of the control operations. However, entomological monitoring conducted one year after the conclusion of the SAT operations indicated that whereas the applied strategy failed to eradicate the *Glossina palpalis gambiensis* and *Glossina tachinoides* populations in the target area, it succeeded in maintaining a high level of suppression (Adam et al., 2013). The failure to achieve eradication was attributed to an insufficient penetration of the insecticide droplets in the dense riparian vegetation during various SAT cycles, a patchy application of the other control techniques, and a strong re-invasion pressure. Within the framework of this campaign and accompanying field operations, we conducted a population genetic study in the intervention area to assess the dispersal capabilities and isolation status of the target populations.

Population genetic studies can be used to estimate dispersal between various populations of tsetse flies (De Meeûs et al., 2007; Gooding and Krafsur, 2005), and as such, they provide a very useful tool to assist decision-making (e.g., to identify sites for the deployment of artificial barriers against re-infestation (Politzar and Cuisance, 1983)) or to facilitate the selection of eradication or suppression strategies depending on the level of isolation of the target population (Solano et al., 2009, 2010a,b).

In the UWR of Ghana, the three main rivers (Black Volta, Kulpawn and Sissili) are characterized by varying but generally increasing density of riverine vegetation along a north–south, downstream gradient. Riverine tsetse populations inhabit these riparian forests and their abundance is modulated by the ecotype of vegetation and its degree of disturbance (Bouyer et al., 2005; Cecchi et al., 2008). The pre-intervention baseline data survey revealed the presence of two riverine *Glossina* species in the region: *G. tachinoides* and *G. p. gambiensis* (Adam et al., 2012). *G. tachinoides* was predominant (98% of all captures) and widespread along all three main rivers, with population densities increasing from north to south. *G. p. gambiensis* was only detected at low densities in the southernmost end of the target area (Adam et al., 2012).

Previous population genetic studies of *G. tachinoides* in Burkina Faso, upstream on the Black Volta River, did not reveal strong structuring within or between populations from different river basins (Koné et al., 2010, 2011). Populations of *G. p. gambiensis* in Burkina Faso appeared more structured than those of *G. tachinoides* (Bouyer et al., 2007, 2009, 2010a; Koné et al., 2011), but data on gene flow indicated that both spec ies were able to disperse between river basins. The capability of riverine flies to cross watersheds was also demonstrated by a mark-release-recapture study with sterile *G. p. gambiensis* in Mali (Vreysen et al., 2013).

Genetic structuring of tsetse populations in the UWR of Ghana has not been studied before. The riparian vegetation along the three main rivers is heavily fragmented, and their tributaries become largely unsuitable for riverine tsetse during the dry season (December–May). Bush fires and the absence of rain during this season seriously limit the availability of favorable vegetation (Adam et al., 2012), so that tsetse flies retract from the tributaries to residual spots along the main rivers, where permanent water and vegetation mitigate the harsh ambient macroclimatic conditions (e.g., temperatures often rise above 40 °C). Whilst the Black Volta River is a permanent water course, the Kulpawn and Sissili are seasonal. It was therefore hypothesized that it might be more challenging for tsetse to disperse between the main rivers in the UWR of Ghana than between the Mouhoun and adjacent river basins in Burkina Faso.

The objective of the present study was to determine whether tsetse populations in the UWR of Ghana were able to disperse between river basins, which were used as the basic operational units for the SAT operations, and hence, whether the tsetse populations of the target area (Adam et al., 2013) could be considered as isolated from adjacent north-eastern populations. The study focused on *G. tachinoides*. The results of this study can be used by managers of the eradication campaign to inform decisions on the potential need to establish adequate barriers to prevent re-invasion of areas where tsetse populations were reduced to very low levels.

2. Materials and methods

2.1. Study area

The Black Volta, Kulpawn and Sissili Rivers are the three main rivers in the UWR. Eight sites located along the three rivers were investigated (Fig. 1). The selected sites had been previously studied in terms of tsetse species composition and abundance (Adam et al., 2012).

2.2. Entomological sampling

From 19/01/2010 to 12/02/2010, 20 georeferenced biconical traps were deployed in each site. On average 5 flies were sampled per trap and a total of 1054 flies were trapped, i.e., 884 *G. tachinoides*, 166 *G. palpalis gambiensis* and 4 *G. morsitans submorsitans*.

2.3. Genotyping

A total of 266 individuals (130 in Black Volta, 44 in Kulpawn and 92 in Sissili) were used for the genetic analysis at microsatellite loci (Table 1 & Fig. 1). The objective was to genotype at least 30 flies per site.

A diagnostic PCR was used to confirm the species (*G. tachinoides*) based on length differences of internal transcribed spacer 1 sequences was performed before genotyping (Dyer et al., 2008).

Nine microsatellite loci were used (preceded by "X" for X-linked loci): XpGp13, pGp24, pGp17, XpGp20, pGp28 and pGp29 (Luna et al., 2001), XB104, C102 (A. Robinson, FAO/IAEA, pers. com.), GpCAG133 (Baker and Krafsur, 2001). In each 1.5 ml tube containing three legs of the tsetse flies, 200 µl of 5% Chelex chelating resin was added (Solano et al., 2000; Walsh et al., 1991). After incubation at 56 °C for one hour, DNA was denatured at 95 °C for 30 min. The tubes were then centrifuged at 12,000 g for two minutes and frozen for later analysis.

The PCR reactions were carried out in a thermocycler (MJ Research, Cambridge, UK) in 20 µl final volume, using 10 µl of the supernatant from the extraction step. The composition of mix solution for each sample was: 2 µl of 10× Buffer, 0.4 µl of 10 mM

Please cite this article in press as: Adam, Y., et al. Genetic comparison of *Glossina tachinoides* populations in three river basins of the Upper West Region of Ghana and implications for tsetse control. Infect. Genet. Evol. (2014), http://dx.doi.org/10.1016/j.meegid.2014.03.023