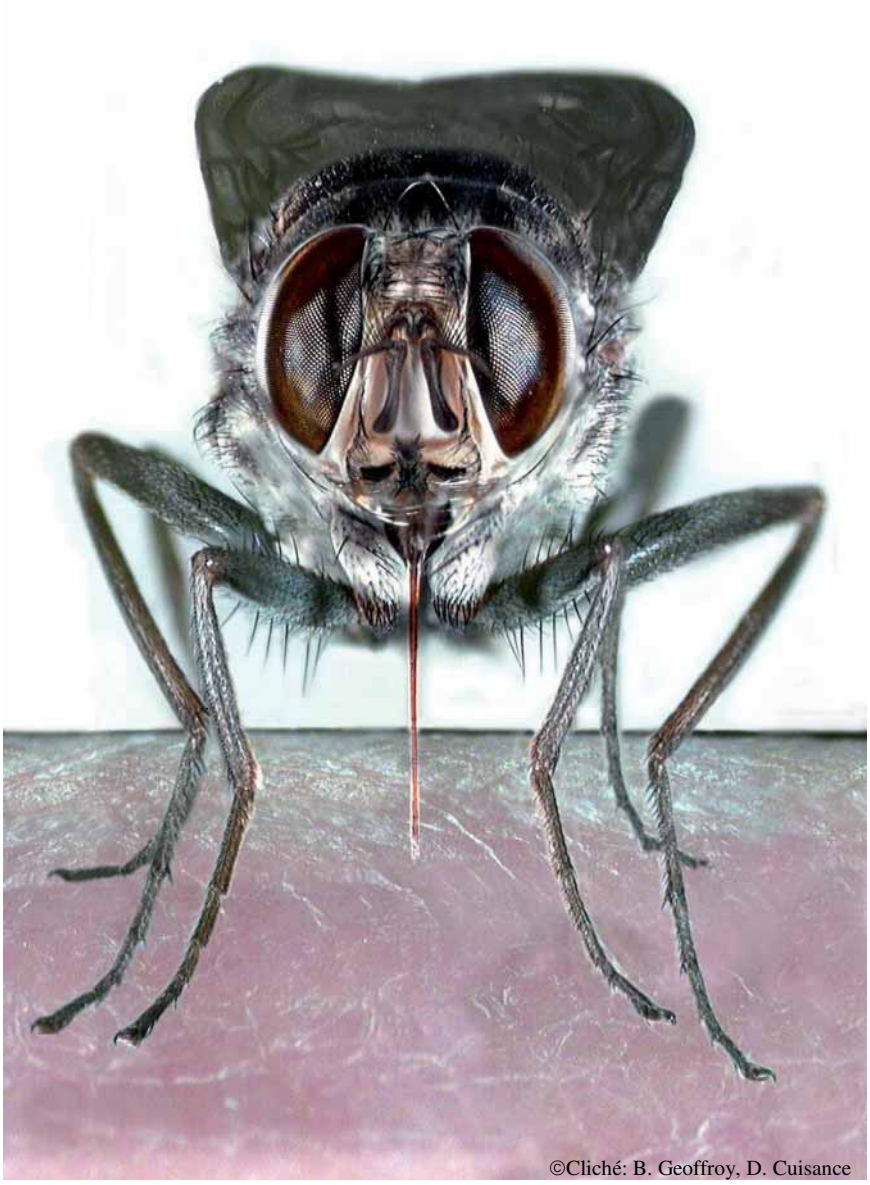
UNIVERSITE MONTPELLIER 2- Habilitation à Diriger les Recherches

Génétique des populations de tsé-tsé et évolution spatio-temporelle des Trypanosomoses



Philippe SOLANO, CR1 IRD, UMR IRD-CIRAD 177, CIRDES Bobo-Dioulasso, BURKINA FASO

Le 07 novembre 2008

Composition du jury :

Prof M.J. LEHANE, Liverpool School of Tropical Medicine and Hygiene, UK- Rapporteur
Prof P. DORCHIES, Ecole Nationale Vétérinaire Toulouse, FRANCE- Rapporteur
Prof P. VAN den BOSSCHE, Institut de Médecine Tropicale Anvers- BELGIQUE- Rapporteur
Prof B. GODELLE, Université Montpellier 2- FRANCE - examinateur
Prof G. DUVALLET, Université Montpellier 3- FRANCE- examinateur
Dr. G. CUNY, Institut de Recherche pour le Développement- FRANCE- examinateur

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1. Curriculum vitae

Etat civil
Philippe SOLANO
Né le 15 avril 1970 à Dakar, Sénégal
Nationalités : Français et Suisse
Marié, 1 enfant

Cursus Universitaire

- **1987** : Baccalauréat Série C, Ecole Française Saint Exupéry, Brazzaville, RP CONGO
- **1990** : DEUG B, Université Bordeaux 1.
- **1991** : Licence de Biologie des Organismes, Université Montpellier 2.
- **1992** : Maîtrise de Biologie des Organismes et des Populations, Université Montpellier 2. Mémoire bibliographique : « Introduction à la biogéographie, la spécificité, et l'écologie des parasites de poissons marins tropicaux : les Monogènes de Carangidae. »
- **1993** : DEA de Parasitologie Université Montpellier 2, « Identification des espèces vectrices de la maladie de Chagas dans le genre *Rhodnius*. »
- **1998** : Thèse de Doctorat de l'Université Montpellier 2, Mention Très Honorable avec Félicitations du Jury. «Implications épidémiologiques de la variabilité génétique des populations de glossines en Afrique de l'Ouest : exemple de *Glossina palpalis* au Burkina Faso»

Cursus professionnel

- **1993** : DEA de Parasitologie, ORSTOM, encadrement : J.P. DUJARDIN, M. TIBAYRENC. *Génétique des populations de triatomes, vecteurs de la maladie de Chagas en Amérique du Sud.*
- **1994-1995** : CSN au CIRDES de Bobo-Dioulasso (Burkina Faso) pour le CIRAD-EMVT, responsables G. DUVALLET, D. CUISANCE. *Caractérisation moléculaire des trypanosomes chez les tsé-tsé.*
- **1996-1998** : thèse Université Montpellier II. Encadrement CIRAD-EMVT (G. DUVALLET, D. CUISANCE), ORSTOM (G. CUNY), CNRS (T. de MEEUS). Responsable de service au CIRDES (2 techniciens, 1 csn). *Génétique des populations de Glossina palpalis au Burkina Faso, Epidémiologie moléculaire des trypanosomoses animales, Amélioration du diagnostic des trypanosomoses animales.*
- **1999** : recrutement à l'IRD (UR 035 Trypanosomoses africaines, resp. G. CUNY), Montpellier. *Génétique des populations de Glossina palpalis, Epidémiologie moléculaire des trypanosomoses animales, Manipulation des parasites par leurs vecteurs.*
- Novembre **1999** : affecté à l'Institut Pierre Richet (IPR) de Bouaké au sein de l'URL-THA dirigée par A. GARCIA.
- **Juillet 2000-septembre 2002** : Responsable de l'Unité de Recherche « THA et glossines » de l'Institut Pierre Richet, 17 personnes dont 3 chercheurs, 3 étudiants en thèse. *Génétique des populations de Glossina palpalis en Côte d'Ivoire, Amélioration du diagnostic de la Trypanosomose Humaine Africaine, Epidémiologie moléculaire et spatiale de la THA.*
- **Septembre 2002- Mars 2005**, Suite aux évènements de septembre 2002 en Côte

d'Ivoire, déménagement forcé de Bouaké à Abidjan. Délocalisation de l'IPR à Abidjan, transfert du matériel. Passage CR2-CR1 en 2003.

- Depuis septembre 2004, en plus de mes activités de chercheur, j'assure de manière permanente l'intérim de la représentation de l'IRD en Côte d'Ivoire (jusqu'en avril 2008), avec notamment l'évacuation des français liés de près ou de loin à l'IRD en novembre 2004. Gestion de 15 personnels permanents, environ 60 personnes accueillies sur le Centre, gestion du partenariat, du budget de la représentation et du Centre IRD de Petit Bassam.
- **01 avril 2005**, nouvelle affectation à l'IRD Bobo-Dioulasso, BURKINA FASO.
- Depuis **Février 2006**, affectation au CIRDES Bobo-Dioulasso, BURKINA FASO. L'IRD est un nouveau partenaire pour le CIRDES où nous sommes accueillis via une convention d'accueil. Obtention en 2006 de divers financements de projets dont UE INCO-DEV, Fondation B&M Gates, FSP REFS. Le CIRDES devient partenaire principal de l'OMS pour la surveillance de la THA en Afrique de l'Ouest.

Compétences

- Génétique des Populations de vecteurs (Trypanosomoses Africaines et Américaines)
- Diagnostic, Epidémiologie Moléculaire et Spatiale des Trypanosomoses Africaines (Humaine et Animales)
- Coordination de réseaux, Expertise, Surveillance Epidémiologique THA
- Représentant p.i. IRD en Côte d'Ivoire (sept 2004- avril 2008)
- Membre élu du conseil d'unité de l'UMR 177 IRD-CIRAD depuis novembre 2007

Régions d'expérience

- Europe : France (Montpellier, Bordeaux)
- Afrique sub-saharienne : Burkina Faso, Côte d'Ivoire, Tchad, Congo (longue durée) ; Guinée, Sénégal, Mali, Zimbabwe, Kenya, Afrique du Sud, RDC, Bénin (courtes durées).
- Amérique du Sud, Asie : Brésil, Vietnam (courtes durées).

Manifestations/Initiatives Scientifiques

- Co-coordonnateur Réseau LTTRN (Leverhulme Trust Tsetse Research Network, réseau d'appui au PATTEC par la recherche en Génétique des populations) depuis 2005.
- Organisation et co-organisation de réunions scientifiques et d'ateliers internationaux du LTTRN (Montpellier, février 2006; Montpellier avril 2007; Conakry décembre 2007).
- Co-Editeur des actes des réunions LTTRN (CD ROM 2005, 2007).
- Chairman Session « African Trypanosomosis », MEEGID7/EMOP, Valencia, Espana, juillet 2004.
- Membre du Comité d'Organisation de l'atelier Corus « Santé humaine et animale », Bobo-Dioulasso, 24-29/09/2007 ; de l'atelier « Impact des changements climatiques sur les Interactions Elevage Environnement », Niamey, 11-15/02/2008.
- Co-Présidence du symposium ouest africain sur les Biotechnologies, Bobo-Dioulasso, février 2007.
- Co-Présidence du Comité d'Organisation et du Comité Scientifique de la Conférence Internationale « Evolutions démographiques et changements climatiques : impacts sur les maladies transmises par les Vecteurs », Cotonou, 24-27 novembre 2008.
- Conférence de fin de Master MIE (Master International d'Entomologie médicale et

vétérinaire, Université Cotonou Abomey-Calavi et Montpellier 2), invitation, Cotonou, juin 2008.
- Chairman Symposium "Génétique des populations de vecteurs", EMOP X, Paris, 25-29 août 2008.

Distinctions

Premier prix du poster scientifique aux VII° actualités du Pharo, "Les fièvres hémorragiques virales et communications libres en pathologie tropicale", 08-09/09/2000 : Intérêt de la génétique moléculaire des tsé-tsé dans l'épidémiologie et le contrôle des trypanosomoses africaines (cas de *Glossina palpalis gambiensis*).

Sociétés savantes

- Membre de la Société Française de Parasitologie
- Membre du Réseau Epidémiologie & Développement (RED)

Expertise

- Referee pour les revues « *Trends in Parasitology* », « *PLoS Neglected Tropical Diseases* », « *Revue d'Elevage et de Médecine vétérinaire des pays Tropicaux* », « *Acta tropica* », « *Biochemichal genetics* », « *International Journal of Tropical Insect Science* », « *Veterinary Research* », « *Parasites and Vectors* », « *Acta tropica* ».
- Expert du programme AIRES-SUD (2008).
- Referee pour demandes de financement au Wellcome Trust (1999, 2005)
- External reviewer pour Comité Entomologie Moléculaire de OMS-TDR (2006)
- Consultances pour l'AIEA (Agence Internationale de l'Energie Atomique) : 7-10j/an depuis 2002
- Membre du Comité Exécutif OMS/TDR pour le séquençage du génome de la tsé-tsé depuis 2004 (IGGI : International *Glossina* Genomics Initiative).
- Missions OMS (surveillance épidémiologique, programmes nationaux de lutte THA), environ 10j/an depuis 2002.
- Missions OMS des chercheurs et techniciens de notre équipe THA en Afrique de l'Ouest (« Equipe d'appui aux Programmes Nationaux »), à l'IPR de 2000 à 2005, puis au CIRDES depuis 2005 (Surveillance THA au Mali, Guinée, Côte d'Ivoire, Burkina Faso, Bénin).

Autres Informations

- Collaborateur du chapitre « Faune sauvage » de l'Atlas du Bassin du Lac Tchad (1997, CIRAD-EMVT/CTA)
- Collaborateur de l'ouvrage « faune sauvage du complexe W-Arly-Pendjari », 2004, CIRAD.

2. Liste des publications

DEA, Thèse

- Implications épidémiologiques de la variabilité génétique des populations de glossines en Afrique de l'Ouest : exemple de *Glossina palpalis* au Burkina Faso. Thèse de Doctorat, Université Montpellier 2, 1998.
- Identification des espèces vectrices de la maladie de Chagas dans le genre *Rhodnius*. Rapport de DEA, Université Montpellier 2, 1993.

Ouvrages, chapitres d'ouvrage

- Solano P., Bouyer J, Itard J. & Cuisance D. (2008). Cyclical vectors of Trypanosomosis. In Infectious and Parasitic Diseases of Livestock, Chap 012. P.C. Lefèvre, J. Chermette, R. Blancou & G. Uilenberg Eds., Editions Lavoisier Tec&Doc, in press.
- Desquesnes M., Itard J., CunyG., Solano P. & Authié E. (2008). Trypanosomoses : Diagnostic. In Infectious and Parasitic Diseases of Livestock, Chap 126. P.C. Lefèvre, J. Chermette, R. Blancou & G. Uilenberg Eds., Editions Lavoisier Tec&Doc, in press.
- Bouyer J., Solano P., Cuisance D, Itard J., Frézil J.L., & Authié E. (2008). Control methods in Trypanosomosis. In Infectious and Parasitic Diseases of Livestock, Chap 127. P.C. Lefèvre, J. Chermette, R. Blancou & G. Uilenberg Eds., Editions Lavoisier Tec&Doc, in press.
- Desquesnes M., Itard J., Cuny G., Solano P., Authié E. (2003). Trypanosomoses. Diagnostic (chap 123). *In*: Principales maladies infectieuses et parasitaires du bétail. Europe et régions chaudes. Lefèvre P.C., Blancou J. & Chermette R. Editions Tec & Doc, Editions Médicales Internationales, 2 tomes, 1747 pages.
- Cuisance D., Itard J., Solano P., Desquesnes M., Frézil J.L. & Authié E. (2003). Trypanosomoses. Méthodes de lutte (chap 124). *In* Principales maladies infectieuses et parasitaires du bétail. Europe et régions chaudes. Lefèvre P.C., Blancou J. & Chermette R. Editions Tec & Doc, Editions Médicales Internationales, 2 tomes, 1747 pages.
- de La Rocque S., Michel J.F., Cuisance D., de Wispelaere G., Solano P., Augusseau X., Arnaud M., Guillobez S. (2001). Le risque trypanosomien : une approche globale pour une décision locale. Du satellite au microsatellite. CIRAD, 151 pages.

Publications scientifiques de rang A (revues internationales à comité de lecture)

2007-2008

- Solano P., S. Ravel, J. Bouyer, M. Camara, M.S. Kagbadouno, N. Dyer, L. Gardes, D. Herault, M.J. Donnelly & T. De Meeûs. Population Structures of Insular and Continental *Glossina palpalis gambiensis* in Littoral Guinea. PLoS NTD, submitted.
- Dyer N.A., S.P. Lawton, S. Ravel, K.S. Choi, M.J. Lehane, A. Robinson, L.M. Okedi, M. Hall, P.Solano and M. J. Donnelly (2008). Molecular phylogenetics of tsetse flies (*Diptera: Glossinidae*) based on mitochondrial (CO1, 16S, ND2) and nuclear ribosomal DNA sequences, with an emphasis on the *palpalis* group. Mol. Phylogenetics and Evolution, accepted. DOI: 10.1016/j.ympev.2008.07.011
- *1. Courtin F., Jamonneau V., Duvallet G., Garcia A., Coulibaly B., Cuny G. & P. Solano (2008). Sleeping sickness in West Africa (1906-2006): Changes in spatial

repartition and lessons from the past. Tropical Medicine and International Health, 13, 1-11. [1]

- *2. Kagbadouno M., Camara M., J. Bouyer, J.P. Hervouet, O. Morifaso, D. Kaba, V. Jamonneau & P. Solano. Tsetse elimination: its interest and feasibility in the historical sleeping sickness focus of Loos islands, Guinea. Parasite, soumis.
- 3. Holzmuller P., Courtois P., Koffi M., Biron D. G., Bras-Gonçalves R., Daulouède S., Solano P., Cuny G., Vincendeau P. & Jamonneau V (2008). Virulence and pathogenicity patterns of *Trypanosoma brucei gambiense* field isolates in experimentally infected mouse: differences in host immune response modulation by secretome and proteomics. *Microbes and Infections*, 10, 79-86.
- 4. Bouyer J, Ravel S., Dujardin J.P., de Meeüs T., Vial L., Thévenon S., Guerrini L., Sidibé I., & P. Solano (2007). Population structuring of *Glossina palpalis gambiensis* (Diptera: Glossinidae) according to landscape fragmentation in the Mouhoun river, Burkina Faso. J. Med. Entomol., 44, 788-795.
- *5. Koffi M., Solano P., Barnabé C., de Meeûs T., Cuny G. & Jamonneau V (2007). Genetic characterisation of *T. brucei s.l.* by microsatellite typing: new perspectives for the molecular epidemiology of human African trypanosomosis. Infection, Genetics and Evolution, 7, 684-695.
- 6. Ravel S., De Meeus T., Dujardin J.P., Zézé D.G., Gooding R.H., Sane B., Dusfour I., G. Cuny & P. Solano (2007). Different genetic groups occur within *Glossina palpalis palpalis* in the sleeping sickness focus of Bonon, Côte d'Ivoire. Infection, Genetics and Evolution, 7, 116-125.

2006

- *7. Camara M., Caro-Riano H., Ravel S., Dujardin J.P., Hervouet J.P., de Meeüs T., Kagbadouno M., Bouyer J. & P. Solano (2006). Genetic and morphometric evidence for population isolation of *Glossina palpalis gambiensis* from Loos islands, Guinea. J Med Entomol., 43, 853-860.
- 8. Garcia A., D. Courtin, M. Koffi, P. Solano, V. Jamonneau (2006). Human African Trypanosomiasis : connecting parasite and host genetics. Trends in Parasitology, 22, 405-409.
- *9. Koffi M., P. Solano, M. Denizot, H. Hebraud, D. Courtin, A. Garcia, V. Lejon, P. Büscher, G. Cuny & V. Jamonneau (2006). Aparasitemic serological suspects in Human African trypanosomiasis: a potential human reservoir of parasites? Acta tropica 98, 183-188.
- *10. Kaba D., Dje N.N., Courtin F., Oke E., Koffi M., Garcia A., Jamonneau V. & P. Solano (2006). L'impact de la guerre sur l'évolution de la THA dans le Centre-Ouest de la Côte d'Ivoire. Trop Med International Health 11, 136-143.
- 11. Lejon V., Jamonneau V., Solano P., Atchade P., Mumba D., Nkoy N., Bébronne N., Kibonja T., Balharbi F., Wierckx A., Boelaert M. and Büscher P (2006). Detection of trypanosome specific antibodies in saliva, towards non-invasive serological diagnosis of sleeping sickness. Trop Med International Health, 11, 620-627.

2005

- *12. Courtin F., Jamonneau V., Oké E., Oswald Y., Coulibaly B., Dupont S., Doumenge J.P., Cuny G. & P. Solano (2005). Trying to understand presence/absence of Human African Trypanosomiasis in Côte d'Ivoire: analysis of the actors of the pathogenic system. International Journal of Health Geographics, 4:27.

*: sont marquées d'une astérisque les publications où un étudiant encadré est premier auteur

- *13. Courtin F., Dupont S., Zeze D.G., Jamonneau V., Sané B., Coulibaly B., Cuny G. & P. Solano (2005). Trypanosomose Humaine Africaine : transmission urbaine dans le foyer de Bonon (Côte d'Ivoire). Trop Med International Health, 10, 340-346.
- *14. Camara M., Kaba D., Kagbadouno M., Sanon J.R., Ouendeno F. & P. Solano (2005). La Trypanosomose Humaine Africaine en zone de mangrove en Guinée : caractéristiques épidémiologiques et cliniques de deux foyers voisins. Médecine Tropicale, 65, 155-161.

2004

- 15. Gooding R.H., Solano P., Ravel S. (2004). X chromosome mapping experiments suggest occurrence of cryptic species in the tsetse fly, *Glossina palpalis palpalis* (Diptera: Glossinidae). Can. J. Zool., 82, 1902-1909.
- 16. Jamonneau V., S. Ravel, M. Koffi, D. Zeze, D. Kaba, L. N'Dri, B. Coulibaly, G. Cuny & P. Solano (2004). Mixed trypanosome infections in tsetse and pigs and their epidemiological significance in a sleeping sickness focus in Côte d'Ivoire. Parasitology, 129, 693-702.
- 17. Jamonneau V., S. Ravel, A. Garcia, M. Koffi, C. Laveissière, S. Herder, P. Grébaut, G. Cuny & P. Solano (2004). Genetic characterization of trypanosomes infecting untreated but asymptomatic sleeping sickness patients in Côte d'Ivoire: a new genetic group? Ann. Trop. Med. Parasitol., 98, 329-337.

2003

- 18. Solano P., A. Koné, A. Garcia, B. Sané, V. Michel, J.F. Michel, B. Coulibaly, V. Jamonneau, D. Kaba, S. Dupont & F. Fournet (2003). Rôle des déplacements des malades dans l'épidémiologie de la Trypanosomose Humaine Africaine dans le foyer de Bonon, Côte d'Ivoire. Médecine Tropicale, 63, 577-582.
- 19. Jamonneau V., C. Barnabé, M. Koffi, P. N'Guessan, A. Koffi, B. Sané, G. Cuny & P. Solano (2003). Identification of *Trypanosoma brucei* circulating in a sleeping sickness focus in Côte d'Ivoire: assessment of genotype selection by the isolation method. Infection, Genetics and Evolution, 3, 143-149.
- 20. Lejon V., C.J.M. Sindic, M. P. Van Antwerpen, F. Doua, N. Dje, P. Solano, V. Jamonneau, I. Wouters & P. Büscher (2003). Human African trypanosomiasis: Quantitative and qualitative assessment of intrathecal immune response. Eur. J. Neurol., 10, 711-719.
- 21. Jamonneau V., P. Solano, A. Garcia, V. Lejon, N.N. Djé, T.W. Miezan & P. Büscher (2003). Stage determination and therapeutic decision in human African trypanosomiasis: value of PCR and IgM quantification on the cerebrospinal fluid of sleeping sickness patients in Côte d'Ivoire. Trop. Med. International Health, 8, 589-594.

2002

- 22. Michel J.F., S. Dray, S. de La Rocque, G. De Wispelaere, M. Desquesnes, P. Solano, D. Cuisance (2002). Modelling the spatial distribution of bovine trypanosomosis within a mixed crop-livestock system area in Burkina Faso. Preventive Veterinary Medicine, 56, 5-18.
- 23. Jamonneau V., A. Garcia, S. Ravel, G. Cuny, B. Oury, P. Solano, P. N'Guessan, L. N'Dri, R. Sanon, J.L. Frézil & P. Truc (2002). Genetic characterisation of *Trypanosoma brucei ssp*. and clinical evolution of Human African Trypanosomiasis in Côte d'Ivoire. Trop. Med. International. Health., 7, 610-621.
- 24. Solano P., V. Jamonneau, P. Nguessan, L. N'Dri, V. Lejon, P. Büscher & A. Garcia (2002). Comparison of different DNA preparation protocols for PCR diagnosis of Human African Trypanosomosis in Côte d'Ivoire. Acta tropica, 82, 349-356.
- 25. De La Rocque S., B. Geoffroy, J.F. Michel, F. Borne, P. Solano, J.Y. Meunier, D.

Cuisance (2002). Les ailes de glossines, une carte d'identité de l'insecte? Parasite 9 (3) : 275-281.

2001

- 26. Jamonneau V., P. Solano, G. Cuny (2001). Utilisation de la biologie moléculaire dans le diagnostic de la trypanosomose Humaine Africaine. Médecine tropicale, 61, 347-354 (*article sollicité*).
- 27. Solano P., JF Guégan, JM Reifenberg, F. Thomas (2001). Trying to identify, predict and explain the presence of african trypanosomes in tsetse flies. J. Parasitol., 87, 1058-1063.

2000

- 28. Solano P., de La Rocque S., de Meeüs T., Cuny G., Duvallet G., & Cuisance D. (2000). Microsatellite DNA markers reveal genetic differentiation among populations of *Glossina palpalis gambiensis* collected in the agropastoral zone of Sideradougou, Burkina Faso. Insect Molecular Biology, 9, 433-439.
- 29. Sané B., Solano P., Garcia A., Fournet F., Laveissière C. (2000). Variations intraspécifiques de la taille des ailes et du thorax chez *Glossina palpalis palpalis* en zone forestière de Côte d'Ivoire. Rev. Elev. Méd.vét. pays Trop., 53, 245-248.

1999

- 30. Solano P., de La Rocque S., Cuisance D., Geoffroy B., de Meeus T., Cuny G. & Duvallet G. (1999). Intraspecific variability in natural populations of *Glossina palpalis gambiensis* from West Africa, revealed by genetic and morphometric analyses. Med. Vet. Entomol., 13, 401-407.
- 31. Solano P., Michel J.F., Lefrançois T., de La Rocque S., Sidibé I., Zoungrana A. & Cuisance D. (1999). Polymerase Chain Reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. Vet. Parasitol., 86, 95-103.
- 32. Duvallet G., de La Rocque S., Reifenberg J.M., Solano P., Lefrançois T., Michel J.F., Bengaly Z., Sidibé I., Cuisance D., Cuny G. (1999). Review on the molecular tools for the understanding of the epidemiology of Animal Trypanosomosis in West Africa. Mem. Inst. Oswaldo Cruz, 94 (2), 245-248.
- 33. Lefrançois T, Solano P, Bauer B, Kabore I, Touré SM, Cuny G., Duvallet G. (1999). Polymerase Chain Reaction characterization of trypanosomes in *Glossina morsitans submorsitans* and *G. tachinoides* collected on the game ranch of Nazinga, Burkina Faso. Acta tropica 72, 65-77.
- 34. Desquesnes M., Michel J.F., de La Rocque S., Solano P., Millogo L., Bengaly Z. & Sidibé I. (1999). Enquête parasitologique et sérologique (ELISA-indirectes) sur les trypanosomoses des bovins dans la zone de Sidéradougou, Burkina Faso. Rev. Elev. Méd.vét. pays Trop., 52, 223-232.
- 35. De La Rocque S., Bengaly Z., Michel J.F., Solano P., Sidibé I. & Cuisance D. (1999). Importance des interfaces spatiales et temporelles entre les bovins et les glossines dans la transmission de la trypanosomose animale en Afrique de l'Ouest. Rev. Elev. Méd. Vét. Pays Trop., 52, 215-222.

1998

- 36. Lefrançois T., Solano P., De la Rocque S., Bengaly Z., Reifenberg J.M., Kabore I. & Cuisance D. (1998). New epidemiological features on animal trypanosomosis by molecular analysis in the pastoral zone of Sideradougou, Burkina Faso. *Molecular Ecology*, 7, 897-904.

1997

- 37. Solano P., Cuny G., Duvallet G., Cuisance D., Ravel S., Sidibé I. & Touré S.M. (1997). Les techniques de génétique moléculaire au service de l'épidémiologie des trypanosomoses - Intérêt de l'étude du polymorphisme des microsatellites des

glossines. Rev. Elev. Méd. Vet. trop, 50 (4), 297-301.

- 38. Solano P., Duvallet G., Dumas V., Cuisance D. & Cuny G. (1997). Microsatellite markers for genetic population studies in *Glossina palpalis* (Diptera: Glossinidae). *Acta Tropica,* 65, 175-180.
- 39. Solano P., Desquesnes M., Sidibe I. (1997). Le diagnostic de *T. vivax* : un problème non résolu dans l'épidémiologie des trypanosomoses. *Rev. Elev. Méd. Vet. trop*, 50 (3), 209-213.
- 40. Reifenberg J.M., Solano P., Bauer B., Kabore I., Cuny G., Duvallet G. & Cuisance D. (1997). Apport de la technique PCR pour une meilleure compréhension de l'épizootiologie des trypanosomoses bovines : exemple de la zone d'aménagement pastoral de Yalé au Burkina Faso. *Rev. Elev. Méd. Vet. trop*, 50 (1), 14-22.
- 41. Reifenberg J.M., Solano P., Duvallet G., Cuisance D., Simpore J. & Cuny G. (1997). Molecular characterization of trypanosomes isolates from naturally infected animals in Burkina Faso. *Veterinary Parasitology*, 71, 251-262.

1996

- 42. Solano P., Dujardin J.P., Schofield C.J., Romana C. & Tibayrenc M. (1996). Isoenzymes as a tool for identification of *Rhodnius* species. *Research & Reviews in Parasitology*, 56 (1), 41-47.
- 43. Solano P., Reifenberg J.M., Amsler-Delafosse S., Kabore I., Cuisance D. & Duvallet G. (1996). Trypanosome characterization by polymerase chain reaction in *Glossina palpalis gambiensis* and *G. tachinoides* from Burkina Faso. *Medical and Veterinary Entomology*, 10, 354-358.

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- 1. Solano P., Camara M., Kagbadouno M., Bouyer J., Hervouet J.P., Ravel S. & Schofield C.J. (2007). Campagne d'élimination des tsé-tsé sur les îles de Loos. LTTRN meeting, Kassa (Rép Guinée), 09-14 déc 2007.
- 2. Ravel S. & Solano P. (2007). Genetic studies on *Glossina palpalis gambiensis* in République de Guinée : evidence for population isolation. LTTRN meeting, Kassa (Rép. Guinée), 09-14 déc 2007.
- 3. Hervouet J.P., Kagbadouno M., Bouyer J., Camara M. & Solano P. (2007). Les îles de Loos et la Trypanosomiase Humaine Africaine : enquêtes préliminaires. LTTRN meeting, Kassa (Rép Guinée), 09-14 déc 2007.
- 4. Ravel S., Wincker P., Solano P., de Meeüs T., Dujardin J.P., Bouyer J. & Cuny G. (2006). Update on *G. palpalis* genomics at IRD/Genoscope. WHO/TDR executive commitee meeting on International *Glossina* Genomics Initiative, Cambridge, UK, 15-17 dec 2006.
- 5. Solano P. Winker S. Aksoy, A. Viari, R. Frutos, S. Ravel & G. Cuny (2005). Update on *G. palpalis* genomics at IRD/genoscope. WHO/TDR executive commitee meeting on International *Glossina* Genomics Initiative, Washington DC, USA, 15-17 dec 2005.

- 6. Solano P., Ravel S., de Meeüs T., Dujardin J.P. & Cuny G. (2005). Role of tsetse population genetics in disease epidemiology. 54th American Society of Tropical Medicine and Hygiene, Washington DC, USA, 11-15/12/2005, invited paper.
- 7. Solano P., Ravel S., de Meeus T., Bouyer J. & Dujardin J.P (2005). The sampling and processing of tsetse flies to assess gene flow among different populations and its importance for AW-IPM programmes. Invited paper, IAEA consultant meeting, Vienne, Autriche, 10-14/10/2005.
- 8. Solano P., Ravel S., de Meeüs T. & Dujardin J.P. (2005). Structuration génétique des populations de *Glossina palpalis* en Afrique de l'Ouest. Implications sur l'épidémiologie et le contrôle des trypanosomoses africaines. Réseau Epidémiologie & Développement, Paris, 06-07 sept 2005, invité.
- 9. Koffi M., Jamonneau V., N'Dri L., Solano P. (2005). Test of different primers and DNA preparations for the use of the PCR in the diagnosis of sleeping sickness. 3rd FAO/IAEA Research Coordination Meeting on "developing, validating, and standardising methodologies for the use of PCR and PCR-ELISA in the diagnosis and monitoring of control and eradication programmes for trypanosomosis", Hanoi, Vietnam, 20-24/06/2005. Invited paper.
- 10. Solano P. Winker S. Aksoy, A. Viari, R. Frutos & G. Cuny (2005). Update on *G. palpalis* genomics at IRD/genoscope within IGGI. WHO/TDR executive commitee meeting on International Glossina Genomics Initiative, Geneva, 03-04 feb. 2005.
- 11. Solano P., J.P. Dujardin, J. Patterson (2005). Objectives and techniques in tsetse population genetics. Leverhulme Trust Tsetse Research Network (LTTRN) 1st meeting, Addis Abeba, Ethiopia, 05-06 feb 2005.
- 12. Solano P., Jamonneau V., Koffi M., N'Dri L. (2004). Le diagnostic de la THA à *T. b. gambiense* en Afrique de l'Ouest. *Atelier d'élaboration de plans d'action pour l'intensification de la lutte en vue de l'élimination de la THA*, Bamako, Mali, 29/06-02/07/2004. Invited paper.
- 13. Solano P., Kaba D., Jamonneau V., Garcia A., Courtin F. & Cuny G (2004). La collaboration inter-pays : les expériences de l'IRD/IPR en matière de coordination et de lutte contre la THA en Afrique de l'Ouest. *Atelier d'élaboration de plans d'action pour l'intensification de la lutte en vue de l'élimination de la THA*, Bamako, Mali, 29/06-02/07/2004. Invited paper.
- 14. Jamonneau V., M. Denizot, G. Cuny & P. Solano (2003). Diagnostic, stage determination and therapeutic decision in Human African Trypanosomosis : values and limits of the PCR. *Invited paper*, *State of the Art Workshop & Roundtable on "Diagnosis & Treatment of Human African Trypanosomiasis", satellite meeting to the 27th ISCTRC meeting, Pretoria, Afrique du Sud, 02/10/2003*.
- 15. Solano P., S. Ravel, T. De Meeus, S. De La Rocque, B. Sane, D. Zeze, L. Ndri, V. Jamonneau & G. Cuny (2003). Genetic differentiation in natural populations of *Glossina palpalis s.l.* using microsatellite DNA polymorphism : artifacts, demes, or cryptic species? 4th FAO/IAEA Research Coordination meeting on 'genetic application to improve SIT for tsetse control/eradication including population genetics', Edmonton, Canada, 23-27/06/03. *Invited paper.*
- 16. Jamonneau V., P. Solano, A. Garcia, V. Lejon, N. Djé, T.W. Miezan, P. N'Guessan, G. Cuny & P. Büscher (2003). Application of PCR/CSF for stage determination and therapeutic decision in Human African Trypanosomosis in Côte d'Ivoire. 2nd FAO/IAEA Research Coordination Meeting on "developing, validating, and standardising methodologies for the use of PCR and PCR-ELISA in the diagnosis and monitoring of control and eradication programmes for trypanosomosis", Rio de Janeiro, Brésil, 07-11/04/03. *Invited paper.*

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- 18. Solano P., V. Jamonneau, P. Nguessan, L. Ndri, N.N. Dje, T.W. Miezan, V. Lejon, P. Büscher & A. Garcia. Comparison of different DNA preparation protocols for PCR diagnosis of Human African Trypanosomosis in Côte d'Ivoire. Report of 1st FAO/IAEA Research Coordination Meeting on "developing, validating, and standardising methodologies for the use of PCR and PCR-ELISA in the diagnosis and monitoring of control and eradication programmes for trypanosomosis", Anvers, Belgique, 26-30/03/2001. *Invited paper*
- 19. Solano P., S. de La Rocque, G. Cuny, I. Sidibe, D. Cuisance & G. Duvallet (1999). Population genetics in *Glossina palpalis gambiensis* using microsatellite DNA polymorphism. Implication on trypanosomosis epidemiology and control. 2nd Research Coordination meeting on "genetics application to improve the SIT for tsetse control/eradication including population genetics", Mombasa, Kenya, 03-07/10/99.

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Journées de l'école doctorale "Biologie des systèmes intégrés, Agronomie, Environnement." Agropolis International, Montpellier, 19-20/10/95.

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Autres publications, Vulgarisation, autres supports

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Reportage de journaliste : La maladie du sommeil en Côte d'Ivoire. Reportage de Sandra Fontaine, journaliste, après une visite de terrain, Jeune Afrique l'Intelligent, Janvier 2003, 3 p.

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Solano P, Dujardin J.P., Pereira J. & Tibayrenc M. Isozymic versus morphological differences among species of the genus *Rhodnius*. In : Proceedings of the IV Congress of Evolutionary Biology, Montpellier , France, 08/94.

Dujardin J.P., Pereira J., Solano P. & Tibayrenc M. (1994). Les réponses de la génétique des populations aux problèmes soulevés par la lutte contre les vecteurs de la maladie de Chagas. In : *« Pour une épidémiologie totale. ». Comptes Rendus du colloque et des ateliers « Homme, Santé, Tropiques ».* 221 pp. Ed. Jacquemin, SIPAP, 121-125.

Rapports

- Rapport d'activité CSN au CIRDES Bobo-Dioulasso, BURKINA FASO, 1995
- Rapports d'activité annuels au CIRDES 1994, 1995, 1996, 1997, BURKINA FASO
- Rapport fin de projet CNRS PIREVS, 1999
- Rapport final ATP santé-environnement CIRAD, 1999
- Rapport d'activité pour la titularisation IRD, 1999.
- Rapport d'activité de l'équipe « THA et glossines », année 2000.
- Rapport final convention SCAC Abidjan (Convention 95000300, engagement N° 20000687) : Projet de recherche sur « les caractéristiques parasitologiques et entomologiques des foyers de THA de Keniéba (Mali), N'Zérékoré (Guinée) et Bonon (Côte d'Ivoire) : implications épidémiologiques ».
- Rapport d'activité de l'équipe « THA et glossines », année 2001 (Institut Pierre Richet, Côte d'Ivoire).
- Sané B., Solano P., Jamonneau V., Degoga I., Diarra B., A.K. Traoré, F. Koné (2001). Enquête épidémiologique et entomologique dans le foyer de trypanosomose humaine de Kéniéba (République du Mali –avril 2001).
- **05/CPR/RAP/2001** Sané B., Solano P., Jamonneau V., Kaba D., Dje Ngoran N. (2001)**.** Enquête sur la Trypanosomose Humaine Africaine dans la préfecture d'Oumé (Côte d'Ivoire-décembre 2001).
- Rapport d'activité de l'équipe THA et glossines, 1er semestre 2002 (INSP, Côte d'Ivoire).
- Rapport d'activité 2002, MENR France (appel d'offre Microbiologie) « Interactions vecteur/hôtes/parasites dans le foyer de Trypanosomose Humaine Africaine de Bonon, RCI »
- **01/CPR/RAP/2002** Sané B., Camara M., N'Guessan P., Solano P. (2002). Enquête sur la Trypanosomose Humaine Africaine en zone de mangrove des prefectures de Boffa et Dubreka

–Guinee (janvier 2002).

- 04/CPR/RAP/2002 Solano P., Dje Ngoran N., Sane B., Kaba D. (2002). Enquête sur la Trypanosomose Humaine Africaine dans les foyers de Bonon et Sinfra (Côte d'Ivoire- avril 2002).

- Rapport partiel projet IAEA n° RC 11413 : «Use of the PCR for the detection of human trypanosomes", 2002

- Rapport partiel projet IAEA n° RC 11421 : « Génétique des populations de *Glossina palpalis palpalis* dans un foyer de trypanosomose humaine africaine en Côte d'Ivoire. », 2003

- P. Solano & P. Cattand (2003). Trypanosomiase humaine africaine : surveillance et lutte en Guinée : Evaluation de la situation épidémiologique et des activités contre la maladie. Mission OMS, 28 janvier- 07 février 2003.

- Rapports annuels CIRDES 2006, 2007 (et présentations au conseil scientifique CIRDES)

- Rapports annuels projet UE INCO-DEV TFCASS 2006, 2007

- Rapport projet FSP-REFS

3. Liste des stages encadrés et formations dispensées

- Formations courtes

1 étudiant angolais (P.J. Cani), novembre 2000, bio-écologie des tsé-tsé (Institut Pierre Richet Bouaké, Côte d'Ivoire)

Formations de terrain : dépistage et diagnostic de la THA ; formations dispensées aux Programmes Nationaux de Lutte lors de prospections médicales (en général sur financement OMS) ; 2002 Guinée, 2003 Bénin, 2005 Burkina Faso.

- Formations longues

Thèse de Médecine :

- Thèse de Médecine Université Abidjan Cocody, D. KABA 2001 : Impact de la lutte anti-vectorielle dans le foyer de Sinfra sur la situation de la THA.

Maîtrise-DEA-Master

SUD

- DEA Génétique option Amélioration des Espèces animales, Université Abidjan Cocody. Mathurin KOFFI (Côte d'Ivoire) : Etude de la variabilité génétique de *Trypanosoma brucei* dans le foyer de THA de Bonon : mise en évidence d'une sélection de génotypes par les techniques d'isolement. 17 Janvier 2003 (présence dans le jury), mention Assez Bien. Co-encadrement avec V. Jamonneau (IRD UMR 177).
- DEA d'Entomologie Médicale du CEMV (Centre d'Entomologie Médicale et Vétérinaire), Université de Bouaké. Dramane KABA (Côte d'Ivoire) : Etude des glossines vectrices des Trypanosomoses Africaines et Lutte anti-vectorielle au 43° BIMA à Abidjan (Port Bouet), Côte d'Ivoire, soutenu 12 octobre 2006, mention Assez Bien (présence dans le jury).
- Master International d'Entomologie (MIE), Universités Cotonou et Montpellier. Moise KAGBADOUNO (Guinée, agent du PNLTHA Guinée), promotion 2007-2008 (**admis**) : Lutte anti tsé-tsé en zone de mangrove de Guinée. Financement FSP REFS / SCAC Conakry.
- Master de Géographie Université Montpellier 3. Jeremi ROUAMBA (géographe Centre Muraz Bobo-Dioulasso), promotion 2007-2008 : Les trypanosomoses humaine et animales dans la boucle du Mouhoun. Financement SCAC Ouagadougou, mention Très bien.

NORD

- Stage MPH Yale University (Maya Nambisan, Yale University, USA) mai-juin 2001 Génétique des tsé-tsé (Institut Pierre Richet Bouaké, Côte d'Ivoire)
- DEA Géographie de la Santé, Université Paris X Nanterre. Sophie DUPONT : Intérêt d'une approche spatialisée pour la définition du risque trypanosomien en zone forestière ivoirienne : le cas du foyer de Bonon. sept 2002. Classée première DEA. Financement IRD/MAE (VCI). Co-encadrement avec Prof AMAT-ROZE, Paris.
- Maîtrise de Géographie, Université Montpellier 3. Fabrice COURTIN : Trypanosomiase Humaine Africaine : Hiérarchisation des lieux de transmission dans le foyer de Bonon (Côte d'Ivoire). 17 Juin 2003.
- DEA Géographie « Mutations Spatiales », Université Montpellier 3. Fabrice COURTIN : Analyse spatiale de la THA dans un foyer de Côte d'Ivoire : intérêt en terme de recherche et de lutte, 20 sept 2004. Classé 1er ex-aequo. Co-encadrement avec Prof J.P.DOUMENGE, Montpellier.

Thèses d'Université

SUD

- Thèse de Doctorat Université Montpellier 2, Mathurin KOFFI : Caractérisation des trypanosomes circulant dans des foyers de Trypanosomose Humaine Africaine: implications en terme de diagnostic, de pathogénicité et d'épidémiologie. Soutenu Décembre 2006. Financement IRD DSF. Co-encadrement avec Prof. Y. COULIBALY (Univ Abidjan), Dr. V. JAMONNEAU (IRD Montpellier).
- Thèse de Doctorat Université Abidjan Cocody, Dramane KABA : Structure des populations de *Glossina palpalis* s.l. en Afrique de l'Ouest : identification de populations isolées ou isolables pour la lutte contre les tsé-tsé et les trypanosomoses. 1ere inscription 2006, en cours. Financement INCO-DEV TFCASS. Co-encadrement avec Prof E. NGORAN, Abidjan et Dr. J.P. DUJARDIN, IRD Mahidol (Thailande).
- Thèse de Doctorat Université Neuchatel, Jean-Baptiste RAYAISSE : étude des attractifs olfactifs de *Glossina palpalis* en Afrique de l'Ouest. 1ere inscription 2007, en cours. Financement B&M Gates Foundation. Co-encadrement avec Prof. P. GUERIN, Neuchatel.
- Thèse de Doctorat Université Abidjan Cocody, Mamadou CAMARA (coordonnateur PNLTHA Guinée) : Eco-épidémiologie de la THA en mangrove Guinéenne. 1ere inscription 2006, en cours. Financement SCAC Conakry. Co-encadrement avec Prof E. NGORAN, Abidjan.

NORD

- Thèse de Doctorat Université Montpellier 3, Géographie. Fabrice COURTIN : 1900-2007 : Mouvements de populations dans l'espace ivoiro-burkinabé, conséquences socio-géographiques au regard de la maladie du sommeil. Soutenu 19 décembre 2007 (présence dans le jury), mention Très Honorable. Financement IRD/MAE (V.I.) et Fondation Inkerman. Co-encadrement avec Prof J.P. DOUMENGE, Montpellier.

Post-Doctorats

- Vincent Jamonneau, 2001, Institut Pierre Richet Bouaké (CI), 1 an, financement IRD, génétique de *Trypanosoma brucei*.
- Mathurin Koffi, 2007-2008, CIRDES, financement UE INCO-DEV TFCASS, Génétique des populations de tsé-tsé et séquençage du génome de *Glossina*.
- Fabrice Courtin, 2008-2009, financement projet FSP-REFS, « Impacts de la démographie et du climat sur la THA en Afrique de l'Ouest ».

Enseignements dispensés

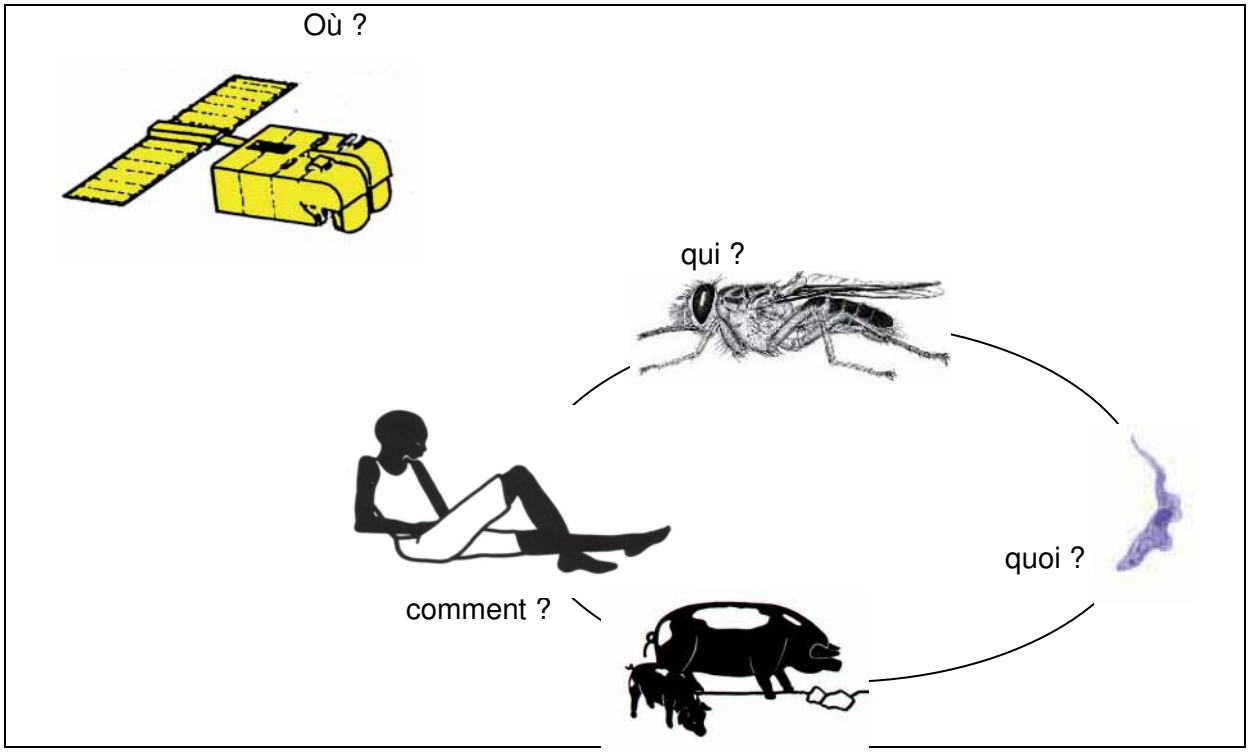
- 4h de cours UFR Sciences Médicales, Bouaké 2001
- 8h de cours DEA Génétique Université Abidjan Cocody 2001
- 12 h de cours DEA Génétique Université Abidjan Cocody 2004 (en 2002-2003 ces cours n'ont pas eu lieu à cause de la crise socio-politique survenue)
- 3 jours de TP/TD Master International d'Entomologie Médicale (MIE Université Cotonou/Montpellier), nov 2006, nov 2007.
- 12h de cours génétique et morphométrie des tsé-tsé, FAO/IAEA training course, Dakar, Sénégal, 10-12 mars 2008.

Formations collectives

- Formation biologie moléculaire : OMS, 2001 Institut Pierre Richet avec S. Ravel (6 participants), LTTRN 2006 Montpellier (15 participants)
- Formation Génétique des Populations avec T. de MEEUS, (CIRDES 2007),
- Formation Morphométrie Géométrique avec J. P. DUJARDIN, CIRDES (2007).

4. Bilan des activités de Recherche

Mes activités de recherche se situent essentiellement dans le domaine de la santé, humaine et/ou animale, et concernent les endémies parasitaires tropicales transmises par les vecteurs, particulièrement les Trypanosomoses humaine (THA ou maladie du sommeil) et animales transmises par les glossines ou mouches tsé-tsé. Le schéma ci-dessous illustre mes intérêts de recherche abordés jusque ici dans le cadre des Trypanosomoses Africaines : **Qui** (*quel vecteur, quelle population, est elle isolée* ?) transmet **quoi** (*caractérisation moléculaire des trypanosomes, génétique, diagnostic*)? **Où** (*spatialisation du risque, SIG*) ? et **Comment** (*modalités de transmission, organisation des hôtes, déplacements de populations*…)? autrement dit, chacun des acteurs du cycle des Trypanosomoses et leurs interactions dans l'environnement.



Diagnostic (TAA et THA) : PCR

Trypanosomoses Animales

La détection de l'infection trypanosomienne sur le terrain repose classiquement sur l'examen microscopique de prélèvements sanguins (techniques de Woo, de Murray), associés à la mesure de l'hématocrite (PCV) ; pour cette dernière il est admis qu'une valeur d'hématocrite inférieure à 25 est synonyme de pathologie. Si l'examen microscopique possède un faible coût et permet d'identifier les sous-genres de trypanosomes, il manque considérablement de sensibilité et beaucoup d'animaux infectés échappent au diagnostic.

Les techniques sérologiques sont le plus souvent appliquées dans les enquêtes conduites sur un grand nombre d'individus; relativement économique, la sérologie anticorps permet de

déterminer le degré de contact des animaux avec le parasite, mais elle ne permet pas la distinction entre différentes espèces de trypanosomes, et ne différencie pas une infection active d'une infection passée. Or, il est indispensable de pouvoir posséder un outil d'aide à la décision qui soit rapide, économique, et fiable, qui s'intègrerait parfaitement dans une approche SIG visant à évaluer le risque trypanosomien dans une zone donnée et proposant une stratégie rapide et adaptée d'intervention. Cet outil diagnostic pourrait aussi être utilisé comme évaluateur de campagnes de contrôle de la trypanosomose.
La technique PCR (Polymerase Chain Reaction) utilisant des séquences d'ADN satellite (répétées) spécifiques d'espèce, ou même de taxons infraspécifiques de trypanosomes offre un compromis sensibilité-spécificité non égalé : des évaluations expérimentales placent sa sensibilité à un ou deux organismes par ml de sang, tandis qu'aucune réaction croisée n'est observée entre les divers groupes de trypanosomes détectés. De plus, la PCR met en évidence l'ADN de trypanosomes, qui disparaît au maximum deux ou trois jours après la mort des parasites. Le test diagnostique donc en majorité des infections actives.
Une étude sur le terrain a été conduite sur des bovins dans la zone de Sidéradougou, au Burkina Faso: l'examen microscopique, et la lecture de l'hématocrite étaient réalisés sur tous les animaux; la PCR a été réalisée sur les animaux positifs au microscope ou dont l'hématocrite était inférieur à 25%. Sur tous les individus ayant montré des trypanosomes au microscope, la PCR en a identifié 90% comme étant des trypanosomes pathogènes. Sur les individus dont l'hématocrite était inférieur à 25%, mais négatifs à l'examen microscopique, 50% ont été trouvés positifs par PCR. Ces résultats ont confirmé que la PCR a une sensibilité supérieure à l'examen microscopique qui aurait entraîné une erreur importante d'appréciation de la prévalence de l'infection trypanosomienne. La PCR présente aussi l'avantage d'une grande spécificité puisqu'on peut distinguer des infections multiples chez un même animal, sans aucune réaction croisée (Solano *et al*., 1999).

Trypanosomose Humaine

La maladie du sommeil évolue en deux phases : la première, lymphatico-sanguine (première période) durant laquelle le parasite se multiplie dans le sang et dans la lymphe, et la deuxième, méningo-encéphalitique (deuxième période) qui correspond au passage du parasite dans le liquide céphalo-rachidien (LCR). Du fait d'une importante diversité clinique, il est difficile d'établir un diagnostic de THA à partir d'un examen clinique seul. Les méthodes de diagnostic indirect, utilisées en dépistage de routine se basent sur la détection d'anticorps ou d'antigènes spécifiques (Exemple : CATT *T. b. gambiense*, Magnus *et al*., 1978). En cas de positivité de l'un de ces tests, le parasite devra être mis en évidence par une méthode directe pour décider du traitement. Le diagnostic direct pour la mise en évidence des trypanosomes consiste en l'observation microscopique de la lymphe et du sang. A l'instar de ce qui se passe en diagnostic des TAA, l'observation du sang, du fait des parasitémies souvent très faibles, nécessite l'utilisation de techniques de concentration comme par exemple, la colonne échangeuse d'anions (mAECt). L'examen du liquide céphalo-rachidien (LCR) permet de déterminer le stade de la maladie (diagnostic de phase), et conditionne la nature du traitement à administrer. Dans ce dernier cas, la recherche de trypanosomes se fait classiquement par simple ou double centrifugation du LCR. Ces techniques étant peu sensibles, des critères indirects d'altération du LCR sont aussi utilisés, comme la protéinorachie (dosage des protéines) et la cytorachie (comptage des leucocytes). Il faut préciser que les médicaments utilisés pour les malades en première période (pentamidine, suramine) sont considérés peu toxiques, alors que le mélarsoprol utilisé pour la seconde période, est un dérivé arsenical avec de forts effets secondaires, responsable d'environ 10% d'encéphalopathies réactives parmi les patients traités.

A partir d'une prospection médicale effectuée à Bonon (Centre Ouest de la Côte d'Ivoire) en 2000, portant sur 13000 personnes, et qui a permis de mettre en évidence 170 séropositifs et 74 malades, plusieurs protocoles de préparation d'ADN à partir du sang ont été testés afin de voir lequel donnait les meilleurs résultats en PCR, avec comme objectif d'améliorer la sensibilité tout en gardant une bonne spécificité, en prenant en compte la reproductibilité et la faisabilité de la méthode.

Quel que soit le protocole utilisé (sang total ou buffy-coat), j'ai montré que la PCR sur sang semble être généralement beaucoup plus sensible que les techniques parasitologiques. La PCR permet aussi de détecter précocement les infections trypanosomiennes à la différence des techniques sérologiques, comme l'ont montrées plusieurs études expérimentales. Dans l'objectif d'avoir un outil diagnostic pour les maladies tropicales, j'ai essayé de considérablement simplifier les protocoles PCR (extraction par résine chélatrice d'anions) en gardant comme priorité de ne pas perdre de sensibilité et de spécificité. Le temps d'exécution et le coût du test ont été réduits, permettant d'appliquer aisément les protocoles dans les conditions de laboratoire de recherche sur le terrain. (Solano *et al*., 2002)

J'ai pu mettre en évidence des problèmes de reproductibilité de la PCR sur des sujets séropositifs mais négatifs à l'examen parasitologique, mais ce type de sujets est celui qui pose le plus de problèmes car aucune méthode n'a pour l'instant permis d'élucider ce phénomène, qui pourrait tout autant refléter des relations hôte-parasite complexes (Garcia *et al*., 2006). D'un point de vue faisabilité, un problème lié à la PCR réside dans le fait que le résultat est extemporané par rapport au prélèvement, à cause du temps d'amplification et de révélation. La PCR, appliquée dans le cadre d'un dépistage de masse, obligerait donc l'équipe de prospection à faire deux passages dans la même zone. Le protocole occasionnerait une perte non négligeable de porteurs de trypanosomes entre les deux passages de l'équipe, ce qui diminue l'impact de la prospection dans le cadre de la lutte contre le réservoir humain. Néanmoins, il est possible de transférer la technique dans des bases de santé rurale pour améliorer le dépistage passif. Une application potentielle à moyen terme, serait de l'utiliser comme le sont actuellement les tests parasitologiques, en confirmation de tests sérologiques lors de prospections actives.

Dans l'optique d'améliorer le diagnostic de phase, les protocoles de PCR sur sang, du fait de leur sensibilité apparente ont été appliqués à la recherche des trypanosomes dans le liquide céphalo-rachidien (LCR).

En termes d'application sur le LCR, nous avons montré que la PCR pouvait contribuer à la décision de classer un malade en première ou seconde phase, ce qui conditionnera son traitement. Ceci est aussi un argument pour équiper les centres de traitement par cet outil. Là encore, il faut s'interroger sur l'objectif à atteindre lorsqu'on pose ce type de diagnostic : a-t-on réellement intérêt à utiliser un test le plus sensible pour le diagnostic de phase, alors que des travaux rapportent que l'on peut traiter avec succès des malades dits de seconde phase à la pentamidine (Doua, 1996)? En revanche, il apparaît un intérêt certain pour le suivi des traitements et le diagnostic précoce des cas de rechute : alors que ces cas de rechute ne pourront être établis que tardivement par les paramètres classiques, la réapparition d'ADN trypanosomien dans le LCR pourra être considérée comme la signature d'une réapparition de la maladie.

Les travaux ci-dessus ont été réalisés dans le cadre du programme international FAO/AIEA appelé « Co-ordinated Research Program on developing, validating, and standardising methodologies for the use of PCR and PCR-ELISA in the diagnosis and monitoring of control and eradication programmes for trypanosomosis. ». J'avais participé à la rédaction de ce programme sur invitation de l'AIEA en 1999, avec G. DUVALLET

(CIRAD-EMVT), R. DWINGER (IAEA Vienne), P. MAJIWA (ILRI), T. LUCKINS (CTVM Edimbourg).

Ces recherches ont constitué une étape parmi d'autres que nous verrons par la suite, qui m'ont fait penser depuis longtemps que les Trypanosomoses Humaine et Animales forment une entité en termes d'objectifs de recherche et de convergence de méthodologies. Améliorer le diagnostic reste un objectif clé à atteindre pour LES trypanosomoses. En matière de trypanosomose animale, la multiplicité des espèces de trypanosomes pathogènes obligeait à multiplier les amorces spécifiques, et nous fumes amenés avec des collègues à envisager une PCR unique, « pan-trypanosomes pathogènes ». A ce moment, j'ai été amené à passer sur le côté « humain » des Trypanosomoses, et certains de mes collègues ont continué de développer ce type d'approches avec des résultats prometteurs (Desquesnes & Davila, 2002), même si ces méthodes ne semblent pas pour l'instant réellement appliquées en dehors des laboratoires de recherche.
En matière de Trypanosomose Humaine, un gros effort financier a récemment vu le jour grâce à la création de la « Fondation of Innovative New Drugs and Diagnostics » (FIND) en partenariat avec l'OMS sur financement B&M Gates Fondation. Récemment, une adaptation de la PCR a été réalisée conduisant à un format plus simple et plus opérationnel, la LAMP (Loop mediated isothermal Amplification of DNA). A la dernière réunion ISCTRC (Luanda, sept 2007), l'adaptation de cette technique au dépistage de la THA même en conditions rurales a été évoquée et semble avoir un avenir prometteur (OMS/FIND, www.finddiagnostics.org/news/newsletters/newsletters_dec_07.pdf).

Epidémiologie moléculaire et spatiale (TAA et THA) :

- *identification moléculaire des trypanosomes chez les vecteurs (BF, CI)*

Les TAA (trypanosomoses animales africaines) ont une importance considérable en Afrique sub-saharienne où elles représentent le principal obstacle au développement de l'élevage. Les trypanosomes, agents étiologiques, sont transmis par les glossines, ou mouches tsé-tsé, aux hôtes vertébrés. Mon objectif, lors de mon séjour de Coopérant du Service national au CIRDES, était de développer une technique permettant de détecter et d'identifier les trypanosomes, et de rechercher des relations privilégiées « taxon de trypanosome-taxon de vecteur ». Pour cela, nous avons d'abord employé la technique de détection par sondes ADN, puis la PCR. Il s'agissait, dans le même temps pour mes collègues et moi, de mener à bien un transfert de technologies visant à installer la PCR, pour la première fois et de manière durable au CIRDES. Je me suis donc intégré dans l'Unité « Epidémiologie et Biotechnologies Appliquées. » où j'avais la responsabilité du service « Biotechnologies Appliquées ». J'y ai adapté la PCR, dans un premier temps pour la détection des trypanosomes chez les glossines. J'ai eu notamment en charge, après des évaluations expérimentales, d'appliquer la PCR à des problématiques de terrain. C'est ainsi que nous avons réalisé plusieurs enquêtes entomo-épidémiologiques dans différents sites au Burkina Faso, mais aussi dans les pays voisins (Côte d'Ivoire, par exemple) : le but était de connaître l'identité précise des trypanosomes trouvés chez les glossines, afin de savoir s'ils représentaient un danger pour l'élevage ou pour la santé humaine. Il faut préciser que la technique classiquement utilisée sur le terrain est la dissection des glossines, mais cette technique présente de sévères limites : faible sensibilité, non détection des infections multiples, identification impossible lorsque des trypanosomes sont présents uniquement dans l'intestin de la glossine, caractérisation des trypanosomes seulement au niveau du sous-genre. La PCR apporte une solution à ces difficultés, et les trypanosomes peuvent être identifiés jusqu'à un niveau taxonomique infra spécifique. Ainsi

dans le sous-genre *Nannomonas*, la PCR permet de distinguer *Trypanosoma simiae*, pathogène chez les suidés, de *T. congolense*, espèce économiquement la plus importante chez les bovins en Afrique. Or ces deux espèces sont morphologiquement semblables chez la glossine, donc indifférenciables par l'examen parasitologique. On comprend l'utilité et la nécessité de développer cette technique si l'on veut comprendre les interactions parasite-vecteur. Nous avons d'ailleurs identifié *T. simiae* chez *G. p. gambiensis* dans le sud du Burkina Faso (Solano *et al*., 1996). Durant ce séjour, nous avons collaboré de manière fructueuse avec l'ILRI (International Livestock Research Institute, Nairobi, Kenya), qui nous a fourni gracieusement les amorces PCR qu'ils avaient mises au point. Les principaux résultats obtenus ont fait l'objet de plusieurs publications et communications, et ont contribué à faire reconnaître le CIRDES comme centre référence de recherche en Afrique, lui assurant un rayonnement régional comme le veut son mandat. A titre d'exemple, en Côte d'Ivoire, *Glossina longipalpis* est trouvée infectée en majorité par *T. congolense* types « savane » et « forêt » (diagnostiqués par une simple PCR), avec beaucoup d'infections matures concomitantes de ces deux types de trypanosomes, dans une zone où la faune sauvage est présente. C'est la première étude qui met en évidence un nombre important d'infections multiples matures chez les glossines en Afrique de l'Ouest, ce qui apporte une nouvelle perception de l'épidémiologie des trypanosomoses. On peut aussi soupçonner un réservoir « faune sauvage » pour ces deux taxons de trypanosomes, dont l'un (le groupe « savane ») est fréquemment trouvé sur les bovins domestiques atteints de trypanosomose. Ce résultat permet aussi la mise en évidence chez un vecteur ubiquiste (*G. longipalpis*), du chevauchement de deux types de cycles, forestier et savanicole, reflétant certainement les modifications écologiques récentes des milieux (Solano *et al*., 1995).

Lors de mon second séjour au CIRDES à l'occasion de ma thèse, j'ai poursuivi des activités sur cette thématique en encadrant un CSN (Thierry Lefrançois, vétérinaire qui a ensuite été recruté au CIRAD). Un premier travail a consisté en la caractérisation par PCR des trypanosomes infectant les glossines (*Glossina tachinoides* et *G. palpalis gambiensis*) dans la zone de Sidéradougou où se conduisait un travail multidisciplinaire sur la spatialisation du risque (de La Rocque *et a*l., 2001). La comparaison parasitologie-PCR est spectaculaire, puisqu'elle montre que 45.5% des infections présumées à *Nannomonas* (par l'examen microscopique), sont en fait des infections à *T. vivax* dans le proboscis associées à une autre infection dans l'intestin, ou des infections à *T. brucei*, ou enfin des infections non identifiées par PCR.
L'utilisation de la PCR a permis de distinguer deux espèces de *Nannomonas* (*T. congolense* et *T. simiae*); la seconde semble absente de la zone de Sidéradougou alors qu'elle est présente chez *G. palpalis gambiensis* 50 km au nord (département de Padéma). La PCR permet aussi de distinguer les formes savane et forêt de *T. congolense* qui semblent avoir des spécificités d'hôtes et des pathogénicités différentes, et enfin de diagnostiquer des infections mixtes (Lefrançois *et al*., 1997; 1998).

- *interactions trypanosome-vecteur*

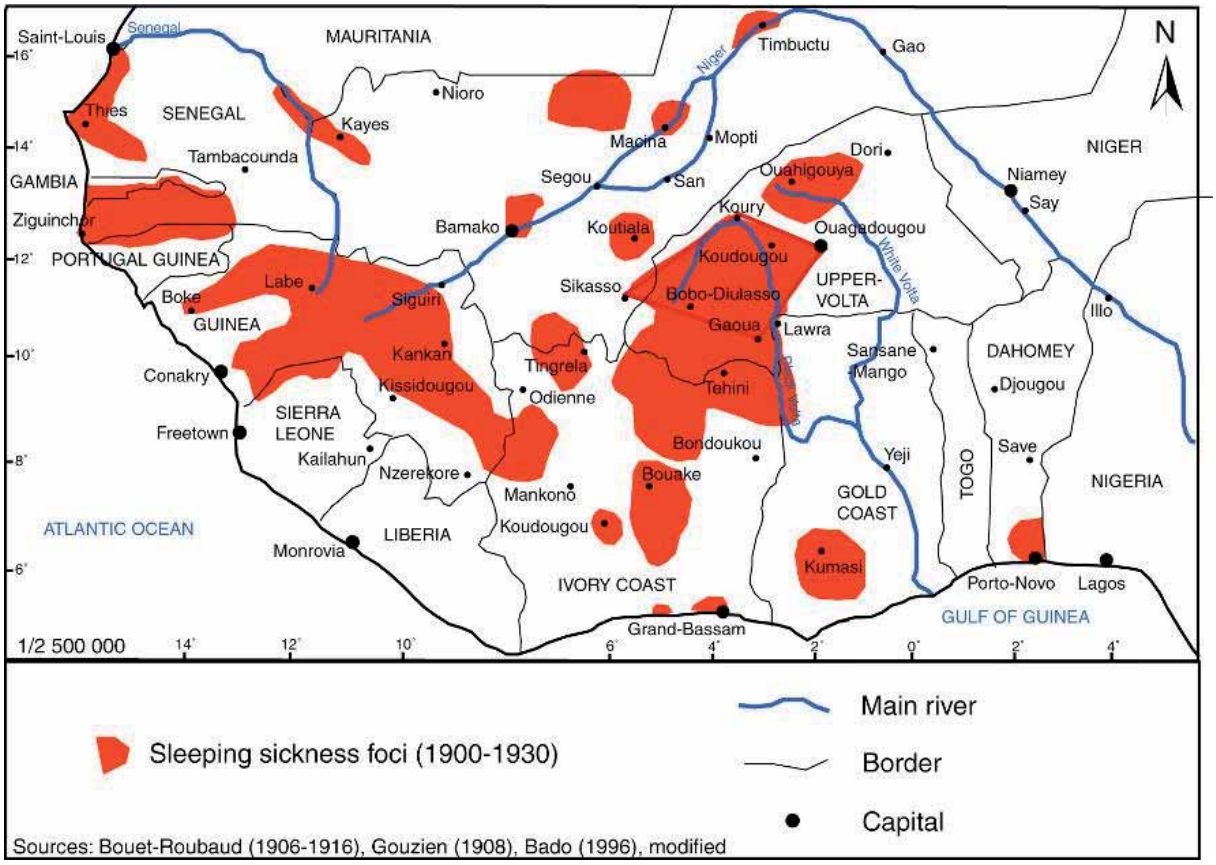
A Montpellier, j'ai repris les données que nous avions accumulées en Afrique de l'Ouest sur l'identification par PCR des trypanosomes chez les tsé-tsé, et je les ai compilées avec d'autres données bibliographiques sur les associations trypanosome-tsé-tsé. Avec des collègues de l'UMR CNRS-IRD dirigée par M. TIBAYRENC (Drs. F. THOMAS, JF GUEGAN), en modélisant grâce à des réseaux de neurones les interactions des variables impliquées dans les associations trypanosome-tsé-tsé (espèce de glossine, répartition géographique, préférences trophiques, types de trypanosomes, etc. etc.), nous avons essayé d'expliquer et de prédire la présence des trypanosomes chez leurs vecteurs par la contribution respective de telle ou telle

variable. Ce travail préliminaire nous a permis de mieux comprendre dans quelle mesure les trypanosomes pouvaient être qualifiés de parasites manipulateurs, en comparaison à d'autres systèmes hôte/parasite, et a aussi permis d'aller plus loin en soupçonnant des phénomènes de « hitch-hiking » parasitaire : lorsqu'un parasite, en l'occurrence *T. congolens*e type forêt en zone de savane, « profite » de la présence d'un parasite manipulateur chez la tsé-tsé (*T. congolense* type savane) pour mener à bien son cycle alors que tout seul, il n'en aurait pas été capable (Solano *et al*., 2001). Cette déduction a été rendue possible par l'utilisation de la PCR pour identifier les trypanosomes chez la tsé-tsé, et le nombre significatif d'infections multiples rencontrées avec ces deux types de trypanosomes chez diverses espèces de tsé-tsé.

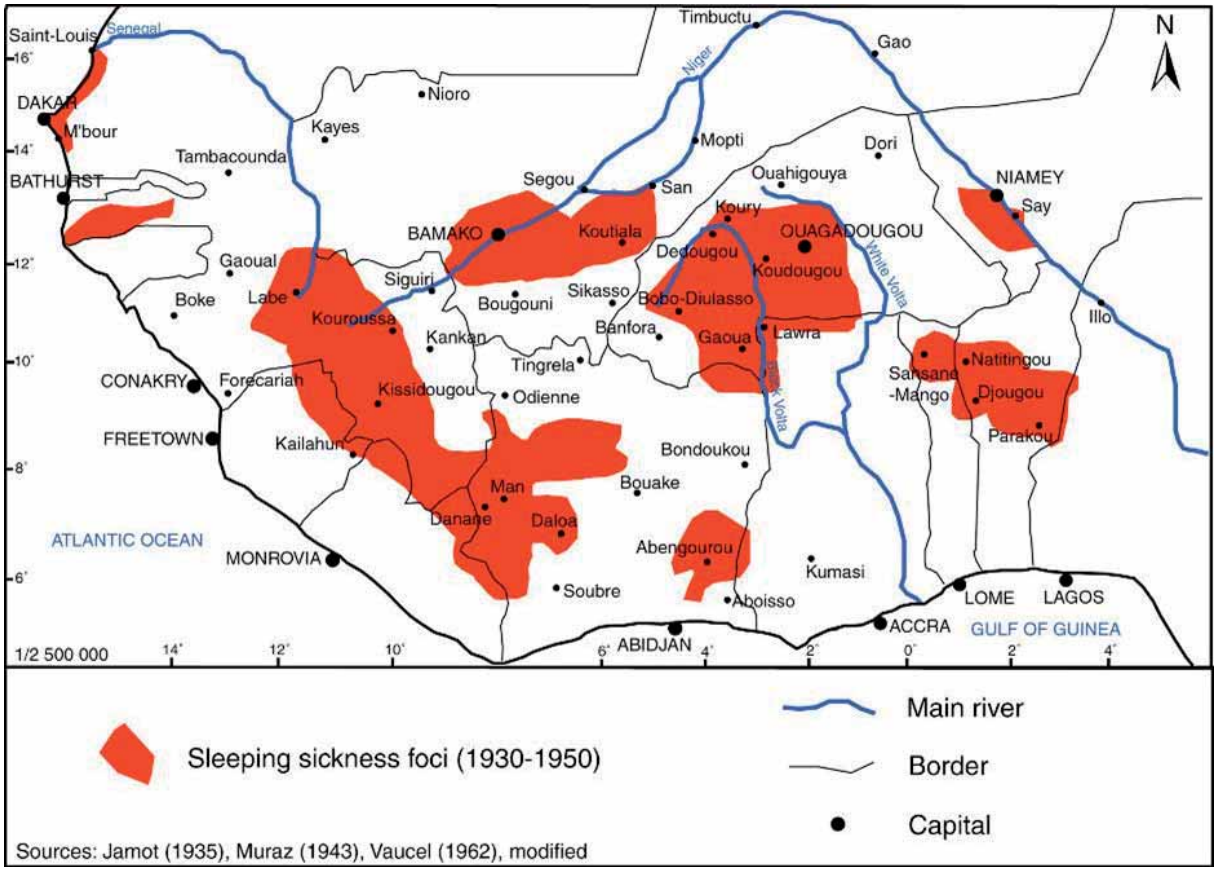
- *Situation THA actuelle, et depuis 1 siècle : lien avec les déplacements de population et les changements climatiques*

Depuis mon arrivée en Côte d'Ivoire, d'abord dans l'équipe de l'IPR, ensuite à Abidjan, puis au CIRDES au Burkina Faso, j'ai toujours souhaité appuyer les équipes des Programmes Nationaux des pays d'Afrique de l'Ouest dans la surveillance de la THA. Cette surveillance est en effet essentielle pour une maladie aussi orpheline que la THA, et les pays n'ont bien souvent ni les moyens financiers, ni les ressources humaines nécessaires pour l'assurer. La surveillance se fait donc essentiellement avec l'appui de l'OMS grâce aux récents financements de la fondation B & M Gates et de Sanofi-Aventis. Lors de nos appuis, la prospection médicale est en général organisée de manière à servir de formation pratique pour l'équipe appuyée : chaque poste (recensement, prélèvement, sérologie, parasitologie, consultation) est doublé dans le cas d'une équipe « nouvelle ». Dans le cas d'une équipe habituée, l'appui peut se focaliser davantage sur tel ou tel poste : c'est souvent le cas du diagnostic parasitologique par la technique de la mini-colonne (mAECt), qui nécessite de la rigueur et de l'expérience. Nous avons été également sollicité pour envoyer soit un technicien, soit un infirmier, appuyer des pays plus éloignés, tels que le Soudan ; nous avons aussi participé à la rédaction d'un document de politique sur la THA (Guinée), qui a constitué l'origine de la création de ce jeune programme de lutte national (2003). Cet appui s'est réalisé progressivement, et une de mes grandes satisfactions est que nous (donc le CIRDES) sommes maintenant considérés par l'OMS comme l'équipe d'appui à la surveillance de la THA en Afrique de l'Ouest.

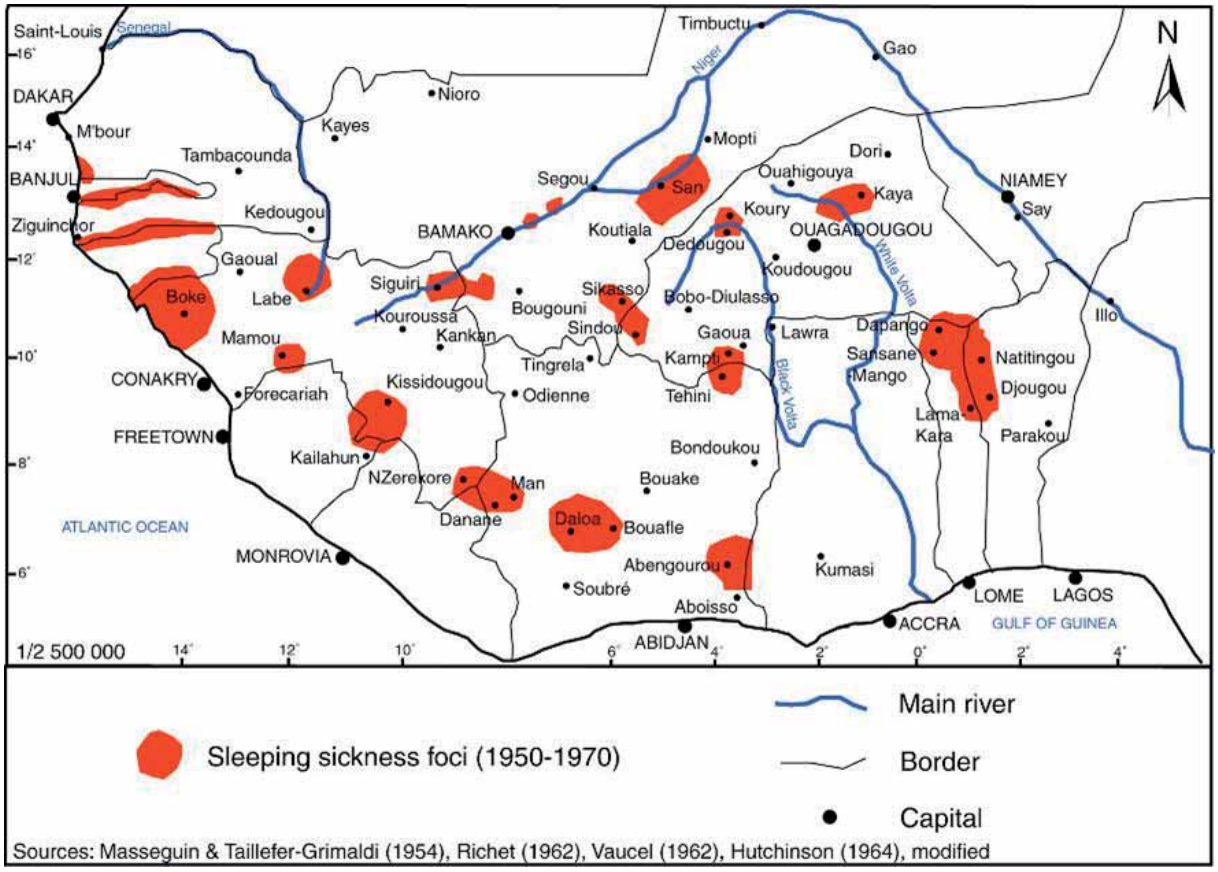
Les figures ci-dessous retracent l'historique de la maladie du sommeil depuis 1 siècle (Fabrice Courtin, Thèse de géographie Université Montpellier 3).



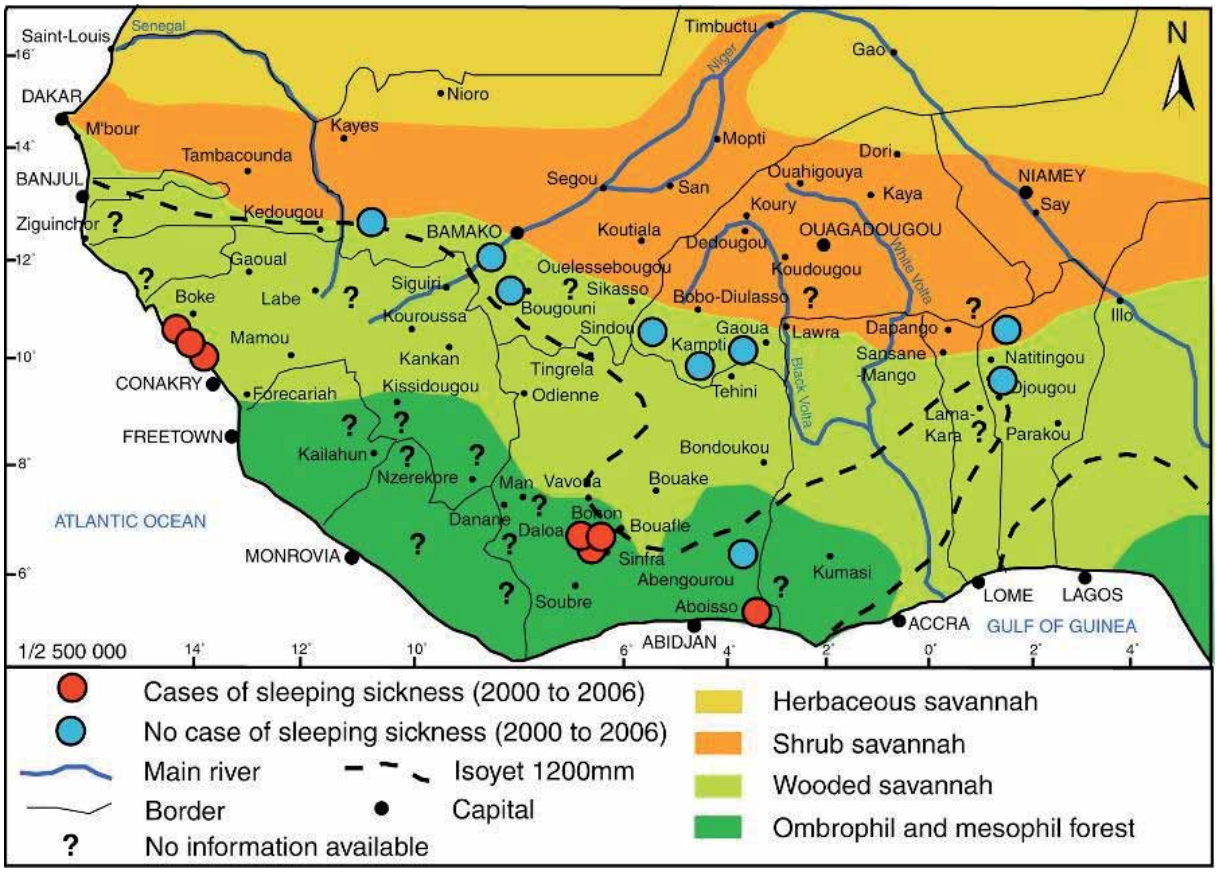
Sources: Bouet-Roubaud (1906-1916), Gouzien (1908), Bado (1996), modified



Sources: Jamot (1935), Muraz (1943), Vaucel (1962), modified

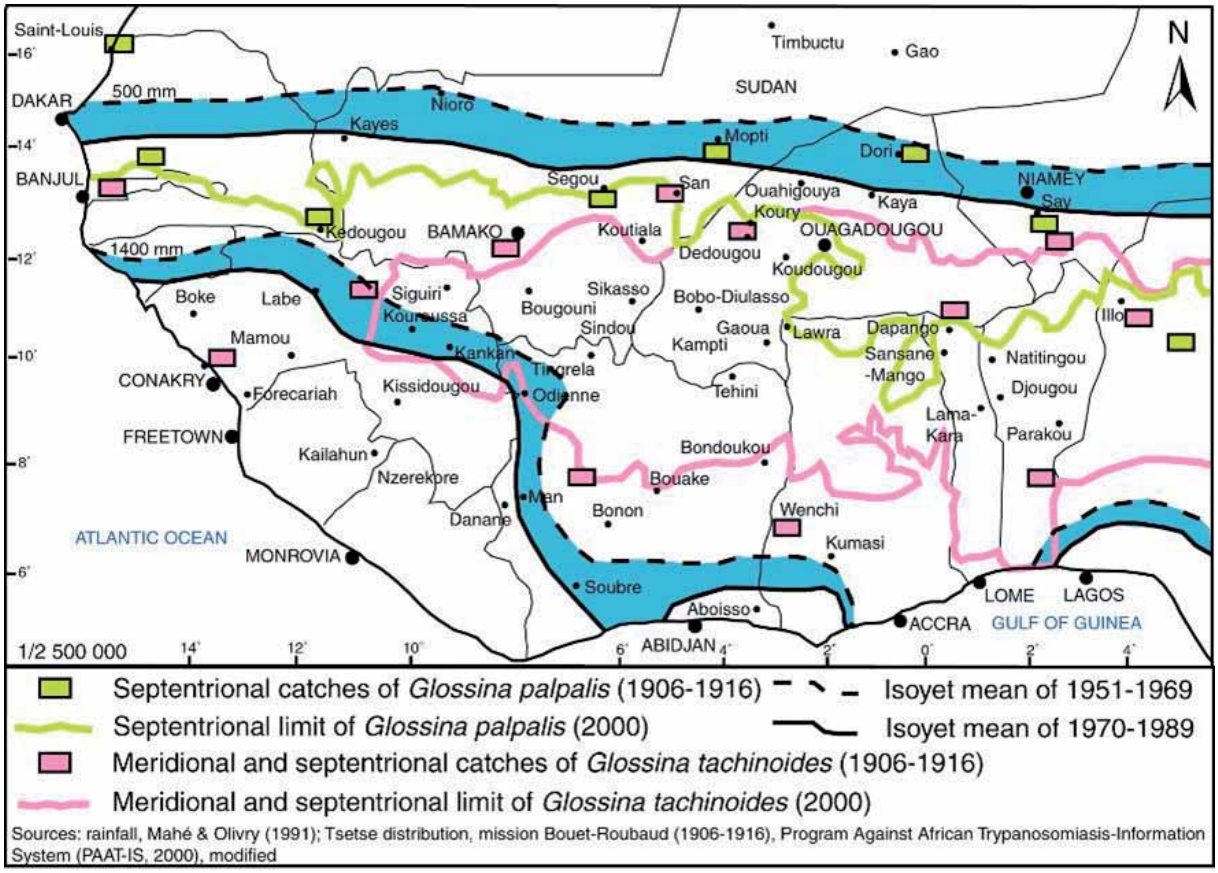


L'examen successif de ces 3 figures montre bien 2 phénomènes : le glissement vers le sud des foyers de THA d'Afrique de l'Ouest, et leur réduction de taille. L'on peut aussi comparer les chiffres de 1939 (250000 cas en Afrique de l'Ouest) et ceux de 2004 (17000 cas officiellement enregistrés par l'OMS pour toute l'Afrique). Le danger serait d'en tirer des conclusions trop hâtives (réduction significative des foyers, du nombre de malades), notamment au vu du nombre de points d'interrogations qui subsistent de nos jours (figure ci-dessous). Ces points d'interrogation illustrent le fait que seulement 10% de la population à risque est actuellement sous surveillance. Si cette maladie semble pouvoir être éliminée grâce à une forte augmentation des efforts de dépistage et de traitement, comme l'annonce l'OMS, il ne faut toutefois pas oublier les leçons de l'histoire, puisque dans les années 1960 on pensait la THA contenue, voire disparue, alors qu'elle a ensuite progressivement ré-émergé pour atteindre une estimation de plus de 300000 cas dans les années 1995-2000 selon l'OMS. L'on ne peut que se réjouir de la volonté affichée de coupler la lutte anti-vectorielle au dépistage médical afin d'atteindre cet objectif d'élimination.



A titre de bilan chiffré, entre 2000 et 2007 depuis l'IPR puis le CIRDES j'ai organisé, ou participé à 25 prospections médicales dans les pays suivants : Côte d'Ivoire, Burkina Faso, Guinée, Bénin, Mali, qui ont concerné environ 120000 personnes et dépisté environ 275 malades qui ont été traités. Je me permets de citer ces chiffres non pas pour affirmer que c'est beaucoup, mais au contraire pour montrer que ces prospections ont été, à une ou deux exceptions près, les seules ayant été organisées en Afrique de l'Ouest durant cette période. La carte de la Figure ci-dessus représente ainsi les résultats des prospections médicales menées entre 2000 et 2006 par les Programmes Nationaux des pays concernés avec notre appui technique et l'appui financier de l'OMS, la coopération française et la coopération belge, avec l'IRD bien sûr.
La situation actuelle telle que nous l'observons est donc la suivante : La THA semble maintenant cantonnée à la zone littorale et forestière d'Afrique de l'Ouest, et ne semble plus exister à l'état endémique en zone de savane (pour ce que nous en savons). Pour visualiser cette répartition, davantage que comme critère d'explication, nous avons proposé l'isohyète 1200 mm comme limite nord de la THA endémique (Courtin *et al*., 2008, TMIH).
Partant de ces observations, la question qui vient immédiatement à l'esprit est : qu'est ce qui explique cette évolution de répartition de la THA avec le temps, en particulier les zones d'extinction et les zones de résurgence ?

Nous n'y répondrons pas ici car évidemment l'explication est très probablement multi-factorielle et nécessiterait un projet multidisciplinaire à elle seule. A titre informatif, quelques facteurs semblent pouvoir déjà être évoqués, tels que bien sûr les opérations de lutte contre la THA de l'époque coloniale qui ont pu stériliser le réservoir humain dans certaines zones, mais aussi très probablement des facteurs climatiques et démographiques avec leurs conséquences sur l'habitat, la distribution des vecteurs, dont certains sont représentés sur la figure suivante.



L'on ne peut s'empêcher de constater que le glissement vers le sud des foyers de THA au cours du siècle se superpose (pas nécessairement aux mêmes échelles et pas nécessairement directement de cause à effet) à un glissement vers le sud des isohyètes (sont représentés ici le 500 et 1500 mm) et de la limite nord de répartition des tsé-tsé. Cette observation, à l'échelle de l'Afrique de l'Ouest, peut comporter des exceptions locales (exemple Dakar où l'on trouve toujours *G. p. gambiensis*) et il sera intéressant de comprendre comment les tsé-tsé arrivent, dans certaines conditions pourtant a priori défavorables, à se maintenir et à maintenir un danger de transmission des trypanosomoses (partie suivante).

Il semble actuellement évident qu'il faille s'intéresser de très près à ces questions pour être capable d'appuyer l'objectif d'élimination et de modéliser de futures évolutions en tenant compte des récentes données mondiales sur le changement climatique global et sur les projections démographiques. C'est l'objet du projet de recherche présenté en fin de document.

Génétique des populations : Réduves et Tsé-Tsé

Les réduves et les tsé-tsé, outre le fait d'être les vecteurs des trypanosomoses, respectivement américaine et africaines, présentent d'autres similarités troublantes : un temps de génération très lent, un temps de vie important à l'état adulte, une faible diversité génétique, et…une absence de développement de résistance aux insecticides, quoi que cela ait été récemment remis en question chez les réduves (Orihuela et al., 2008). (Quand) en sera-t-il de même pour les tsé-tsé ?

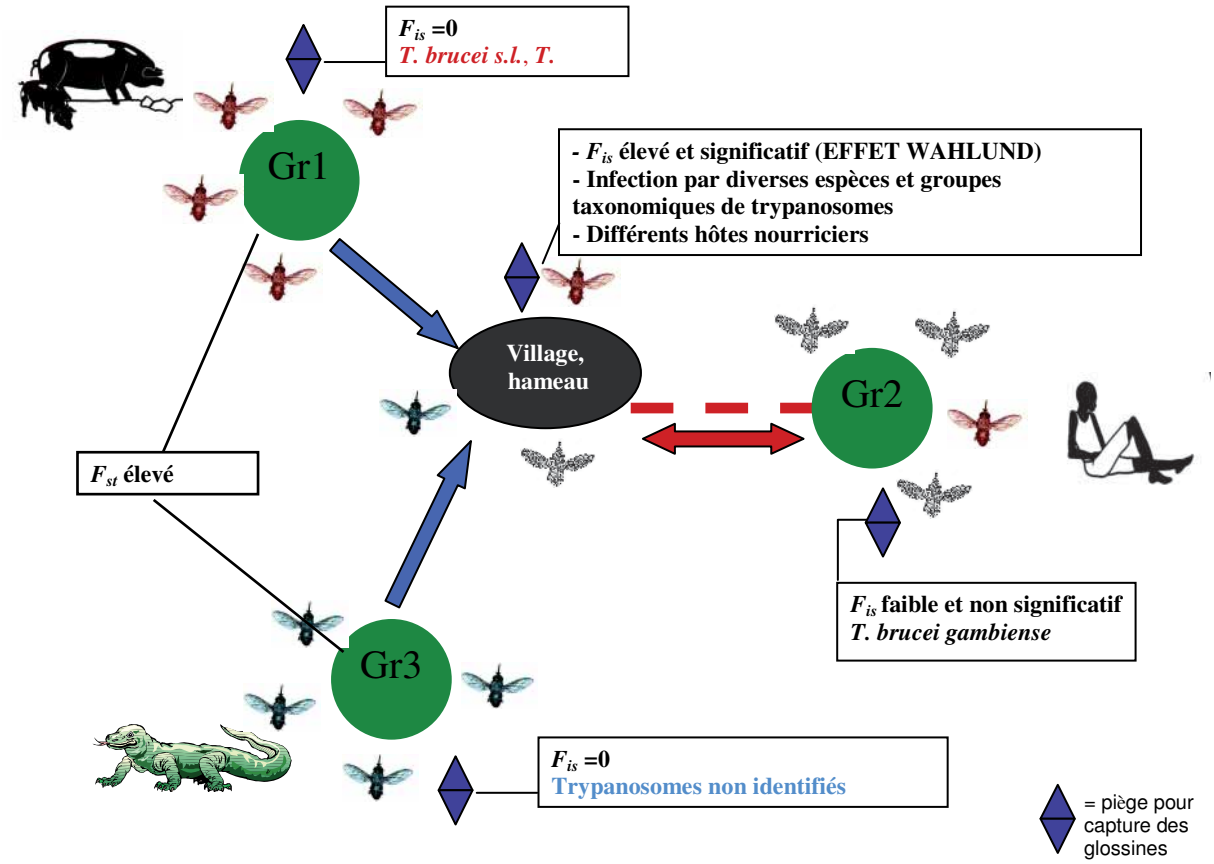
Les punaises du genre *Rhodnius* (Triatominae), vecteurs dont les espèces sont très difficilement reconnaissables morphologiquement entre elles, présentent d'importantes variations de capacité vectorielle de la maladie (trypanosomose américaine ou maladie de Chagas), encore très répandue en Amérique latine. Il est donc important de pouvoir les reconnaître afin d'évaluer le risque de transmission de la maladie lorsqu'on les trouve dans une localité donnée, et de prévoir les réinvasions des zones traitées des espèces ayant un cycle sylvatique. Les résultats principaux obtenus lors de mon DEA ont consisté en l'esquisse d'une clé isoenzymatique de détermination des vecteurs du genre *Rhodnius* (Triatominae) (Solano *et al*., 1996).

En ce qui concerne les tsé-tsé, j'ai eu la chance d'être le premier à identifier des séquences microsatellites chez ce vecteur (Solano *et al*., 1997) lors de ma thèse à partir d'une banque d'ADN génomique total dans le laboratoire de Gérard Cuny, et ai surtout eu la chance que ces loci soient suffisamment polymorphes pour être utilisables en génétique des populations. Nous avons donc utilisé ces marqueurs sur des populations de laboratoire, puis des populations naturelles au Burkina Faso, au Mali et au Sénégal. Un des premiers résultats intéressants a été l'observation de différenciations à échelle macrogéographique entre les *Glossina palpalis gambiensis* du Burkina Faso et du Sénégal (Solano *et al*., 1999). A ces différences génétiques se superposaient des différences morphologiques de la taille des ailes, les glossines du Sénégal étant plus petites que celles du Burkina Faso. Cette différence de taille est compatible avec un état de stress supérieur au Sénégal où la pluviométrie est très faible et où *G. p. gambiensis* se trouve en limite nord et ouest de son aire de répartition.

Mais, au-delà de ces différenciations génétiques entre populations mesurées par Théta, l'estimateur non biaisé du F_{ST} de Weir & Cockerham, 1984 (le F_{ST} est une mesure de la déviation par rapport à la reproduction au hasard entre populations différentes), nous observons des indices du F_{IS} (mesure de la déviation par rapport à la reproduction au hasard au sein des populations, mesuré par son estimateur f) souvent positifs et significatifs, et cela se répètera dans le futur sur des populations de *G. palpalis* s.l. échantillonnées au Burkina Faso (zone de Sidéradougou), en Côte d'Ivoire (foyer de Bonon), en Guinée (îles de Loos, foyer de Dubréka) même si dans ce dernier cas les F_{IS} sont inférieurs à ceux observés au Burkina Faso et en Côte d'Ivoire. Il faut préciser que les toutes premières études étaient réalisées avec seulement 3 loci microsatellites (Solano *et al*., 2000), puis 4 (Camara *et al*., 2006), puis 5 (Ravel *et al*., 2007) et actuellement nous utilisons 8 à 10 loci différents, ce qui permet des interprétations statistiquement plus robustes en génétique des populations (Bouyer *et al*., 2007). Nous avons aussi toujours tenté, lorsque c'était possible d'associer à ces marqueurs moléculaires des marqueurs morphométriques, d'abord sur la taille (Solano *et al*., 1999 ; de La Rocque *et al*., 2002) puis sur la taille combinée à la forme avec les moyens plus récents de la morphométrie géométrique en collaboration avec Jean-Pierre Dujardin (Dujardin & Slice, 2007 ; Camara *et al*., 2006 ; Bouyer *et al*., 2007). En effet, si la taille est directement corrélée à l'environnement puisque les différences de taille chez les tsé-tsé reflètent les conditions de vie de la génération précédente, des changements de forme (d'un organe comme l'aile par exemple) sont évolutivement beaucoup plus « graves » et suggèrent des évolutions génétiques.

Lors des premières analyses de génétique des populations effectuées en collaboration avec T. de Meeüs, nous expliquions les F_{IS} positifs à Sidéradougou par un effet Wahlund (après avoir éliminé la part expliquée par la présence d'allèles nuls et par des phénomènes de « short allele dominance »): les tsé-tsé capturées dans les pièges proviendraient de sous-groupes génétiquement différenciés, qui se retrouveraient groupés artificiellement dans les pièges de capture. Force est de reconnaître que cette première hypothèse émise alors avec peu de marqueurs (Solano *et al*., 2000) est toujours d'actualité, bien qu'elle soit maintenant plus

affinée. En effet cette hypothèse est corroborée par les taux d'infection et les préférences trophiques (toujours analysés chez les mêmes individus, ce qui est un point très important à souligner). Comme à Sidéradougou, où des mouches distantes de quelques kilomètres ont des taux d'infections différents, et des préférences trophiques différentes (Lefrançois *et al.*, 1998), cette tendance se retrouve chez *G. p. palpalis* dans le foyer de THA de Bonon en Côte d'Ivoire (Ravel *et al.*, 2007). Nous illustrons cette interprétation dans la figure suivante.



Sur cette figure sont représentés 3 « groupes » (Gr, chacun représenté par des mouches de couleur différente) génétiquement différenciés de G. palpalis palpalis à Bonon (Côte d'Ivoire). Chacun vit dans son habitat intime (l'ambit de Jackson, 1946) avec des conditions de vie favorables (humidité, hygrométrie), un hôte nourricier principal qui est à l'origine du type d'infection par des trypanosomes (par exemple le porc domestique offre des taux d'infection généralement très élevés par T. congolense et T. brucei en Côte d'Ivoire, les trypanosomes de reptiles ne sont pas reconnus par les marqueurs moléculaires habituels, et l'homme ne peut être infecté que par T. b. gambiense). Or les pièges de capture des glossines, pour avoir des densités maximales, sont généralement posés dans des zones fréquentées par l'homme qui représentent des zones de chasse ou de rencontre pour les glossines. Dans ces pièges se retrouvent donc artificiellement mélangés des groupes ou sous-populations génétiquement différenciées qui expliquent l'effet Wahlund observé. Cette figure illustre le fonctionnement en métapopulations des glossines. Si les pièges étaient posés au sein même de l'ambit de chaque sous-groupe, les Fis observés devraient être beaucoup plus réduits.

Cette interprétation de la structure des populations, développée avec T. de Meeüs, J. Bouyer, et S. Ravel pour *G. p. palpalis* en zone forestière de Côte d'Ivoire, semble applicable aussi pour *G. p. gambiensis* au Burkina Faso. Mais il faut ici préciser qu'en zone de savane sub-humide au Burkina Faso, la dispersion de *G. p. gambiensis*, principalement longitudinale le long des cours d'eau, est beaucoup plus assimilable à un « one dimensional stepping stone model » qu'en zone forestière où les directions de déplacement seront moins focalisées grâce à un réseau hydrographique beaucoup plus dense et des conditions d'hygrométrie moins limitantes. Nous pensons même que ce degré de structuration des populations de tsé-tsé devrait varier en fonction de la fragmentation de l'habitat, ce que nous espérons tester par la suite.
Au Burkina Faso, nous avons donc pu mettre en évidence le long du Mouhoun (rivière principale du pays, qui fait l'objet d'un plan d'élimination des tsé-tsé par le PATTEC) une faible structuration des populations de tsé-tsé sur 260 km (Bouyer *et al*., 2007). Toutefois des F_{IS} élevés sont encore observés au sein de chacune des 4 populations, à des degrés variables mais toujours significatifs. En affinant l'échelle d'analyse, on observe qu'il semble exister un isolement par la distance entre individus d'une même « population ». Il semble donc que pour capturer une population panmictique, il faille réduire les intervalles de piégeage à une distance inférieure à 100m, sous peine de rencontrer un effet Wahlund (T. de Meeüs, com. pers.).
Ces résultats tout récents, dont certains sont encore en cours d'analyse, ont des implications cruciales en terme de lutte anti –tsé-tsé : cette structure des populations de *G. palpalis* en méta-populations, vraisemblablement de petite taille et fortement soumises à des processus densité-dépendants, vivant chacune dans son ambit, contribuerait à expliquer la capacité des tsé-tsé à survivre à des opérations de lutte anti-vectorielle qui ne toucheraient pas la totalité de ces sous-populations, et leur capacité à se re-développer ensuite et à ré-envahir les zones traitées.

Dans ces études, la question posée était le plus souvent : La population échantillonnée est-elle panmictique ? si non pourquoi ?
Avec l'avènement récent de la campagne PATTEC de l'Union Africaine, qui vise à éliminer les tsé-tsé et les Trypanosomoses du continent Africain dans « le temps le plus court possible », j'ai été sollicité avec mes collègues pour répondre à des questions plus « opérationnelles » avec l'outil génétique. L'on ne peut que se réjouir de la prise en compte récente, mais de plus en plus importante, de résultats de génétique des populations dans le choix des stratégies de lutte et leur mise en place. Qui l'eut cru il y a quelques années encore ?
Il faut d'abord rappeler brièvement et reconnaître que la plupart des opérations de lutte anti tsé-tsé, si elles ont été efficaces et ont localement réussi à interrompre la transmission (de la THA comme de la TAA), n'ont pas été durables, et se sont souvent terminées par des réinvasions (ou résurgences), donc des échecs. Une des solutions pour venir en aide au PATTEC serait donc d'intervenir prioritairement sur des populations isolées, ce qui serait une garantie d'efficacité (puisque les méthodes de lutte sont efficaces techniquement parlant) sur le long terme. Population « isolée » peut bien sûr faire penser à des îles, tel l'exemple de Zanzibar où les tsé-tsé ont été éliminées avec succès (Vreysen, 2000). Mais l'on peut aussi penser à des « îles biologiques », et bien sûr c'est là qu'intervient l'outil génétique, qui par la mise en évidence d'absence ou de restriction de flux de gènes entre populations, peut aider à « prioriser » les cibles de la lutte anti-vectorielle dans cet objectif à long terme. C'est tout le sens de la création du réseau LTTRN que je co-coordonne, qui sert ainsi de « bras armé » du PATTEC en génétique des populations. Je donne quelques exemples ci-dessous d'études sur les flux de gènes chez *G. palpalis* avec cet objectif de diagnostiquer un éventuel isolement de

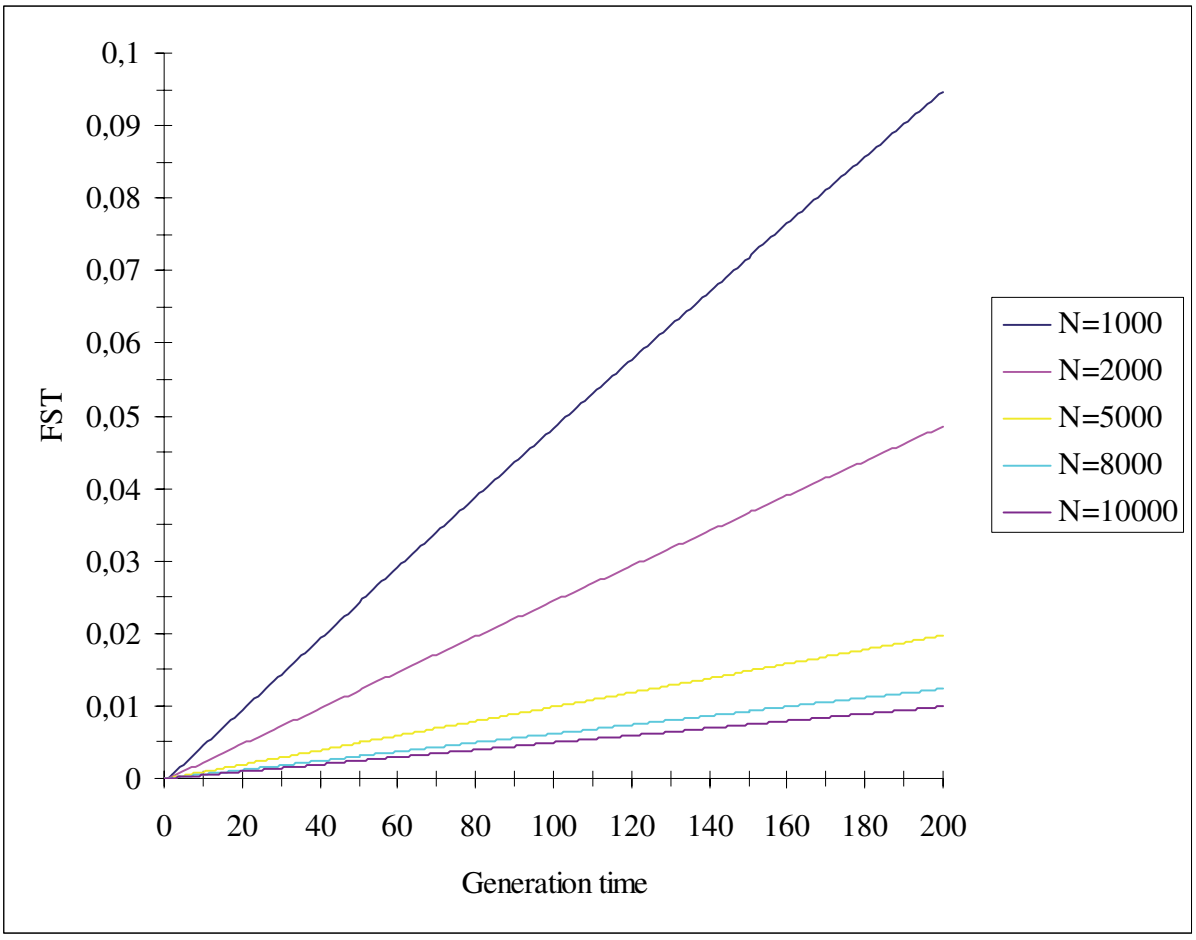
populations.

- En Guinée, les îles de Loos sont à seulement 4 km de la capitale Conakry, présentent l'attrait touristique majeur de la région, hébergent une population humaine d'environ 7000 personnes, et abritent dans le domaine animal une activité économiquement importante d'élevage de porcs domestiques. Ces îles présentent un historique de cas de THA entre les années 1940 et 1980, et sont voisines (30 km) des foyers de THA les plus actifs du pays (Dubréka, sur la zone littorale). Une première enquête a montré la présence de *G. p. gambiensis* sur ces îles en 2005. La question qui s'est immédiatement posée est évidemment : les tsé-tsé de ces îles sont elles isolées de celles des foyers les plus voisins ? Nous avons utilisé les microsatellites et la morphométrie géométrique, qui on concordé pour diagnostiquer un isolement des ces tsé-tsé par rapport à celles du continent (Camara *et al*., 2006). Un fait extrêmement intéressant est que le PNLTHA Guinéen s'est immédiatement saisi de ce résultat de recherche, pour initier une campagne d'élimination des tsé-tsé de ces îles (Kagbadouno et al., 2008 soumis à Parasite). De plus nous avons également montré qu'il y avait une différenciation génétique des populations des différentes îles (3 îles principales), ce qui autorise d'un point de vue intervention, à les attaquer séquentiellement puisqu'il n'y a pas, peu, ou plus d'échange d'individus entre îles. Cette campagne est maintenant en cours avec un appui technique de notre part et de la part des Instituts du sud avec lesquels nous collaborons (IPR Côte d'Ivoire, CIRDES Burkina Faso), et avec le soutien de bailleurs de fonds internationaux (OMS, FSP du MAE français, notre réseau LTTRN) ou privés (Bayer, Vestergaard-Frandsen, Sumitomo). Les premiers résultats sont prometteurs puisqu'en décembre 2007, nous sommes déjà à 99% de réduction sur l'île principale, et les opérations de lutte sur les deux autres îles viennent tout juste d'être mises en place. Ici l'objectif affiché est clairement **l'élimination** à long terme, et les efforts devront continuer jusqu'au bout (jusqu'à la dernière tsé-tsé) sous peine de risque de réinvasion si l'on se « contente » d'arrêter le projet à 99% de réduction. Cette élimination n'est envisageable que si les opérations portent sur une population effectivement isolée (Vreysen et al., 2007). Dans le cas contraire, on préfèrera le **contrôle**, en abaissant les densités (mais sans vouloir les éliminer) à un seuil suffisant pour arrêter la transmission, mais ceci suppose des efforts durables dans le temps.
- Au Burkina Faso dans le cadre du PATTEC, nous analysons par les microsatellites des populations situées sur des réseaux hydrographiques différents, par exemple entre les rivières Comoé et Mouhoun (ex Volta Noire), dans le but de savoir s'il y a ou pas, des échanges de tsé-tsé entre ces bassins. La conséquence est que, soit les tsé-tsé traversent la zone inter-bassin et l'intervention devra tenir compte de cela en installant des barrières de pièges anti tsé-tsé entre les deux bassins, soit elles ne traversent pas et l'intervention sera logistiquement plus simple et plus faisable.
- Ce type d'analyse peut également être réalisé au sein d'un même réseau hydrographique soumis à une fragmentation paysagère, d'origine anthropique souvent (par exemple, mise en place de cultures de coton arrivant en bord de cours d'eau qui vont contribuer à isoler des populations de tsé-tsé, de La Rocque *et al*., 2001). La question devient alors : depuis

combien de temps, et/ou sur combien de kilomètres de réseau les populations de tsé-tsé doivent-elles être séparées pour entraîner une rupture de flux de gènes ? A ce moment tout l'intérêt réside dans la combinaison des analyses paysagères par télédétection et des analyses génétiques afin de localiser ces populations isolées. Le même type d'analyse peut être réalisé dans des zones ayant vu des aménagements tels que des barrages qui vont contribuer à séparer des populations qui auparavant formaient un continuum.

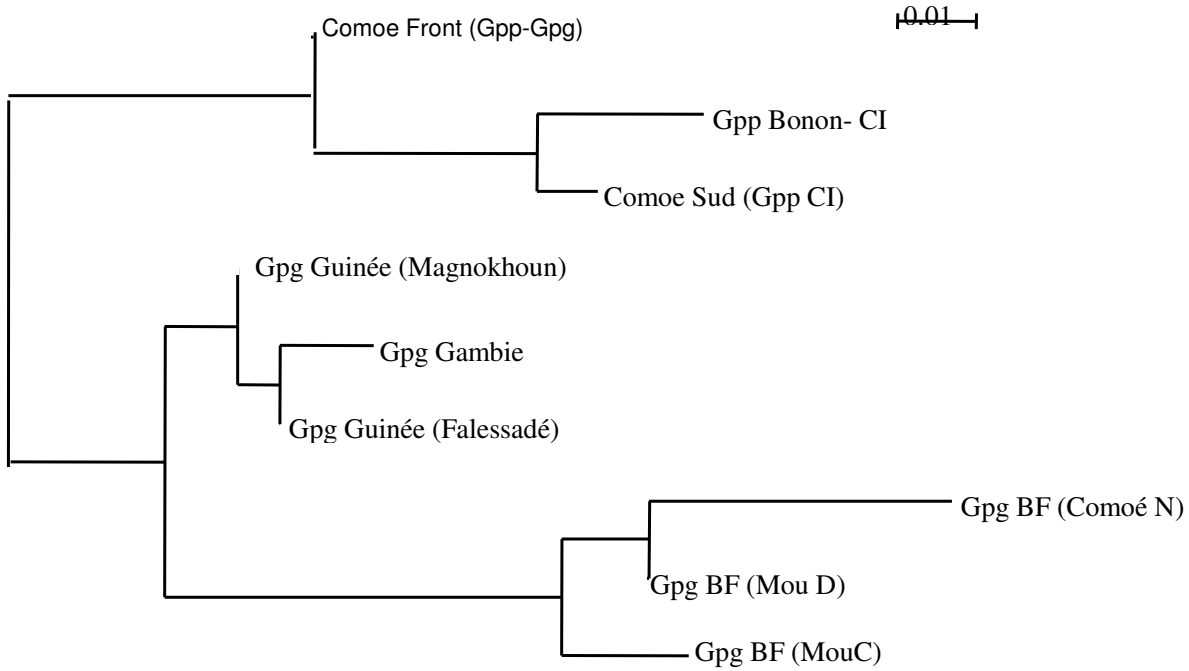
Nous avons commencé à répondre à ce type de questions (O. Esnault, 2007, rapport CEAV ; Bouyer *et al*., en preparation), mais les résultats sont encore très préliminaires pour prétendre donner une réponse opérationnelle claire au PATTEC Burkina Faso. Nous nous en tiendrons à dire ici qu'il semble effectivement possible de détecter des diminutions d'échange génétique entre populations fragmentées d'un même réseau hydrographique, ce qui ne signifie pas pour autant qu'une réinvasion serait impossible. En revanche, d'autres questions apparaissent, notamment sur les outils utilisés pour répondre à ces questions. Une des questions clé à ce sujet semble être : si des populations sont effectivement isolées, nos outils seront-ils capables de le diagnostiquer ? en d'autres termes, combien de temps, de générations de séparation faut-il pour que nos outils détectent cet isolement ?

C'est exactement la question que j'ai posée à Thierry de Meeüs, qui a modélisé l'évolution du F_{ST} (en utilisant les microsatellites) en fonction du nombre de générations depuis lequel 2 populations de taille efficace N sont complètement isolées, adaptant un travail plus ancien de Nei (1975). Le résultat est visualisé dans le graphique suivant.



Ce graphique permet d'illustrer l'exemple suivant : si 2 populations de 2000 individus « efficaces » chacune sont séparée depuis 20 ans (environ 120 générations), le F_{ST} mesuré sera d'environ 0.03.

Enfin, dans le cadre d'un projet Union Européenne récemment obtenu (« TFCASS : Tsetse flies and the Control of African Sleeping Sickness »), je collabore avec la Liverpool School of Tropical Medicine pour faire avancer nos connaissances sur la structure des populations des tsé-tsé du « groupe palpalis » (ou sous-genre *Nemorhina*) à différentes échelles : micro-géographique (structuration locale), macro-géographique (entre bassins versants) pour les études intra-spécifiques, et aussi en inter-spécifique car nous soupçonnons la possibilité d'espèces cryptiques dans ce complexe. Dans un premier temps j'ai tout simplement regroupé, avec la collaboration de Sophie Ravel, dans une même base de données tous les génotypes que l'on a obtenu lors de nos études sur *G. palpalis s.l.* en Afrique de l'Ouest, soit au Burkina Faso, en Guinée, en Côte d'Ivoire et en Gambie (cette dernière dans le cadre d'une prestation de service pour l'AIEA : Agence Internationale de l'Energie Atomique).
La Figure ci-dessous représente donc un « Neighbor Joining tree » basé sur les F_{ST} mesurés par paires de populations. Un autre arbre eut pu être représenté avec les $F_{ST}/(1- F_{ST})$ qui se rapprochent plus d'une véritable distance génétique, ou toute autre distance génétique (Nei, etc). La topographie est la même avec ces différentes représentations, et l'on voit apparaître ici 3 groupes : le premier en partant du haut rassemble les *G. p. palpalis* de Côte d'Ivoire (Bonon, et Aniassué, cette dernière étant localisée sur le fleuve Comoé dans la zone de répartition de *G. p. palpalis*), ainsi qu'une 3ème localité : Gansé, toujours sur le fleuve Comoé, mais exactement à la limite décrite par Challier *et al*., 1983 entre *G. p. palpalis* et *G. p. gambiensis*. En première analyse, nos résultats suggèrent donc qu'actuellement, cette zone comprend une forte majorité de *G. p. palpalis* (F_{ST} Aniassué-Gansé = 0.02 ; F_{ST} Gansé-Folonzo (BF)= 0.18). La valeur du F_{ST} obtenue entre Gansé et une localité située au sud du Burkina Faso, Folonzo, toujours sur ce même fleuve Comoé, est très élevée (0.18), et n'est pas incompatible avec une valeur existant entre deux espèces différentes.
Nos résultats, additionnés à ceux de Gooding, 1991 (lors de croisement Gpg*Gpp, mise en évidence d'hybrides dont les mâles, sexe hétérogamétique, sont stériles, règle de Haldane) et ceux de Challier *et al*., 1983 suggèrent que *G. p. palpalis* et *G. p. gambiensis* constituent deux « bonnes » espèces différentes.
Les *G. p. gambiensis* de notre étude se retrouvent dans le groupe situé au bas de l'arbre, et comprennent des tsé-tsé issues de localités du fleuve Comoé (Folonzo), et du fleuve Mouhoun, deux bassins versants différents.



Enfin, le 3ème groupe, en position intermédiaire sur l'arbre, rassemble des populations de Guinée et de Gambie, pourtant éloignées géographiquement les unes des autres. Représenterait-il les *Glossina palpalis* de « mangrove » d'Afrique de l'Ouest, qui ainsi se distingueraient des autres ? Des études sont en cours pour le confirmer, notamment avec nos collègues de Liverpool qui utilisent du séquençage de marqueurs mitochondriaux (16S, CO1) et nucléaires (EF1, NADH2, ITS2).

Il apparaît que dans les mois ou les années à venir, ces études moléculaires sur les tsé-tsé devraient connaître un nouvel essor avec le séquençage du génome de la tsé-tsé que l'on espère connu fin 2008 début 2009, au moins en partie. L'espèce séquencée, au travers d'un consortium international (IGGI : International *Glossina* Genomics Initiative) sous la bannière de l'OMS/TDR, est *Glossina morsitans*, et notre rôle en collaboration avec Génoscope Paris a été de fournir deux banques d'ADN de *Glossina palpalis* : l'une pour obtenir des EST (13000), l'autre sur des cDNA entiers (environ 1500 gènes). A l'heure actuelle (2008), environ 12000 gènes ont été annotés chez *G. morsitans*, et ceux de *G. palpalis* le seront au premier semestre 2009. Nous espérons ainsi faire grandir la communauté scientifique travaillant sur des aspects évolutifs (notamment comparatifs entre espèces de glossines, mais aussi avec les moustiques, drosophiles), et contribuer à « désisoler » ce vecteur et la THA par conséquent.

Perspectives

Une nouvelle ère s'ouvre vraisemblablement en ce moment pour la recherche sur les tsé-tsé, je suis plutôt d'un naturel optimiste, mais serais prêt à en prendre le pari. Il se trouve que le séquençage du génome de cet organisme sera publié en 2009, à un moment où le changement climatique global pourrait faire penser que les tsé-tsé ne deviendront plus qu'une curiosité épidémiologique dans les années à venir, suite à (selon les scénarios d'évolution climatique actuellement proposés) une restriction de leur aire de distribution due à la baisse de

pluviométrie, l'augmentation des températures (Terblanche *et al*., 2007) et à une anthropisation tellement croissante qu'elle détruira leur habitat (Courtin *et al*., 2008 ; J.P. Guengant & J. May, le Monde 17/12/2007). Mais après tout n'étaient-elles pas déjà considérées comme pas « beaucoup plus » qu'une curiosité ? Il est peut-être temps de rappeler quelques « fondamentaux ». La tsé-tsé est déjà qualifiée depuis longtemps de « fossile vivant dans un anachronisme biologique » (Mortelmans, 1986). Est-ce dû à son si faible taux de reproduction dans la nature qu'on estime qu'une femelle adulte ne donne vie qu'à 2-3 individus viables au long de sa vie ? ou au fait que ses stades larvaires se développent « in utero » jusqu'à être déposés dans le sol dans la nature avant de se transformer en pupe, qui pendant 1 mois ou plus de son développement sera sensible aux inondations, à la prédation, aux changement de température et d'humidité ? ou est ce tout simplement que tout le monde se « désole » que la tsé-tsé n'aie jamais - jusqu'ici - développé de résistance à un quelconque insecticide et y soit si « désespérément » vulnérable qu'on ne comprenne pas pourquoi elle existe encore et pire, cause un des plus grands problèmes à l'Afrique en termes de santé ET d'économie puisqu'elle transmet la Trypanosomose Humaine ET Animale ? D'autant quelle est réputée être un très mauvais vecteur puisque même en zone d'endémie, les taux d'infections de mouches tsé-tsé par le pathogène *T. brucei gambiense* ne dépassent qu'exceptionnellement 0,5 à 1%.

Ce serait peut-être oublier un peu vite que la tsé-tsé (au moins le vecteur principal du groupe palpalis) est capable de prouesses ! Qui eut parié que *Glossina palpalis* put s'adapter tant à l'homme et à son environnement qu'on la trouve dans de grands centres urbains tels que :

- Dakar (400 mm de pluie annuelle), la répartition la plus au nord et la plus à l'Ouest, encore de nos jours, où *G. palpalis* (*gambiensis*) défie toutes les prédictions des entomologistes, climatologues et écologistes, à tel point que l'AIEA vient d'y lancer un projet d'élimination de ce milieu particulier des « Niayes ».
- Abidjan : on ne trouve pas de publications récentes sur la présence de *G. palpalis* (*palpalis*), mais pourtant nous l'avons bien observé de nos yeux, avec notre collègue D. Kaba et son équipe, en plein milieu de la ville dans le zoo (en fortes densités !), mais aussi à l'Université Abobo-Adjamé et dans la forêt du Banco; pour ne point trop s'appesantir sur sa présence au 43° BIMA où elle cause la mort de chiens et chevaux militaires en leur transmettant la trypanosomose animale (Kaba 2006) ! Quid de la THA dans ces environnements ? Personne à l'heure actuelle ne peut le dire.
- On ne pourra pas énumérer dans le détail les autres capacités d'adaptation à l'homme de cette espèce qui devrait en faire 2 (au moins) au vu de la génétique (*G. p. gambiensis* et *G. p. palpalis*), mais rappelons qu'elle est présente aussi à Bamako où elle n'a pas été éliminée malgré un programme de lutte à grande envergure, qu'elle transmet la THA dans et autour des nombreux centres urbains secondaires de Côte d'Ivoire tels que Daloa, Bouaflé, Bonon, qu'elle est toujours responsable de la plus grande prévalence de THA en Guinée (pour l'Afrique de l'Ouest), notamment dans la région littorale proche de la capitale Conakry;
- et que c'est sa très proche « cousine phylogénétique » *Glossina fuscipes quanzensis* qui est responsable des grandes épidémies actuelles de THA telles que celle de Kinshasa (Epidémie depuis 1999 avec plusieurs centaines de cas au sein de la ville de Kinshasa , Ebeja *et al*., 2003), mais aussi dans le reste de la RDC ainsi qu'en Angola et au Tchad, par exemple.

Tout cela n'a pas pour but de « complimenter » ce vecteur mais juste de rappeler ses traits de vie, l'un supplémentaire étant que la tsé-tsé n'est (pourquoi ?) sensible à aucun répulsif

actuellement sur le marché, tout au moins si l'on se fie à ce qui est publié sur le sujet.

Ainsi donc, lorsque l'on entend quelques personnes affirmer qu'il n'y a « plus rien à découvrir » sur la tsé-tsé, se permet-on d'en douter, presqu'en silence, car qui cela intéresse t'il vraiment ? D'un point de vue optimiste, parions que la recherche en entomologie médicale, voire même sur l'ensemble des maladies à vecteurs, aurait beaucoup à perdre sans les tsé-tsé et les Trypanosomoses. L'avenir proche nous le dira : le séquençage du génome de la glossine, attendu pour 2009, devrait faire multiplier rapidement- à l'instar de ce qui s'est passé pour *Anopheles gambiae*- le nombre de publications sur le sujet, et surtout le nombre de scientifiques s'y intéressant –au nord comme, on l'espère, au sud. Nul doute qu'au niveau évolutif dans un premier temps (comparaison entre espèces, entre différents vecteurs, avec les drosophiles aussi), mais surtout en termes de transfert de technologies et de renforcement de capacités vers le sud, ces progrès apporteront réellement un renouveau dans le domaine, en espérant de plus que le peu de laboratoires africains de recherche capables de « suivre » puissent effectivement y être associés et saisir cette opportunité.
Le lecteur comprendra donc qu'il reste un énorme potentiel de recherche sur les tsé-tsé et les maladies qu'elles transmettent, ne dussent-elles pas menacer directement l'Europe comme d'autres pathologies plus «connues» au vu de l'actualité, que sont notamment la grippe aviaire, la « Blue tongue » ou Chikungunya, West Nile…L'on commence tout juste à comprendre comment sont organisées certaines populations de tsé-tsé (Solano *et al*., 1999 ; Solano *et al*., 2000 ; Camara *et al*., 2006 ; Ravel *et al*., 2007 ; Bouyer *et al*., 2007), tout au moins celle de *G. palpalis s.l.* dans divers biotopes d'Afrique de l'Ouest (savane humide avec distribution riveraine au Burkina Faso ; savane guinéenne et forêt dégradée avec distribution plus homogène sur le littoral de Guinée et en forêt dégradée de Côte d'Ivoire). Toutefois beaucoup reste à comprendre sur quelques paramètres clé qui émergent de plus en plus en matière d'épidémiologie des trypanosomoses :

- la notion d'apprentissage, qui si elle est confirmée, ferait que la tsé-tsé retournerait sur l'hôte de son premier repas de sang (Bouyer *et al*., 2007), ce qui a des conséquences épidémiologiques importantes, notamment en ce qui concerne la non-obligation d'un réservoir animal pour entretenir l'endémie (si le repas de la mouche ténérale se fait sur l'homme, elle aura tendance à y retourner, pour ce qui est de la THA) ;
- nos collègues belges ont récemment montré que, contrairement à l'idée classiquement répandue selon laquelle la tsé-tsé ne peut s'infecter qu'à son premier repas de sang (tout au moins pour *T. brucei gambiense*), le fait qu'elle soit affamée peut la conduire à s'infecter à tous les repas de sang et non pas qu'au premier (Kubi *et al*., 2006). Ils vont même plus loin (Akoda et al., données en cours de publication) : un état de stress nutritionnel de la mère se retrouvera chez sa larve qui, une fois émergée, pourra également s'infecter à n'importe quel repas de sang et sera un vecteur plus dangereux que des glossines s'étant nourries normalement. Il pourrait être intéressant de tester ces hypothèses sur le terrain avec l'apport d'outils tels que la morphométrie géométrique pour détecter des populations « stressées ».
- en terme d'attractifs olfactifs, les résultats connus jusqu'ici suggèrent que les composés identifiés pour les glossines du groupe morsitans en Afrique de l'Est ne sont pas aussi efficaces sur les tsé-tsé du groupe palpalis en Afrique de l'Ouest : les recherches ont-elles été mal faites (peu probable), ou cela traduit-il d'autres mécanismes de réponse que l'odeur pour les tsé-tsé du groupe palpalis, qui déjà répondent très différemment aux pièges et aux modes de capture, par rapport aux tsé-tsé savanicoles du groupe

morsitans ?

- Pourquoi existe-t-il cette opposition relatée entre les données écologiques, qui indiquent une grande capacité de dispersion pour les tsé-tsé, et les données génétiques qui montrent une forte structuration locale, comme si les tsé-tsé ne se servaient pas de tout de leur « potentiel » de dispersion, ou qu'elles développaient des comportements particuliers (philopatrie…) ? Cette question, qui se retrouve chez d'autres organismes où l'on observe aussi une apparente contradiction entre données génétiques et écologiques, pourrait trouver son explication dans la notion d'ambit confirmée par nos résultats génétiques tels qu'expliqués pour *G. p. palpalis* en Côte d'Ivoire ;
- Des questions avec des incidences plus opérationnelles se posent ici, que nous avons dans un premier temps soulevé, avant de commencer à y répondre via notre réseau LTRRN et nos actions en Guinée et au Burkina Faso dans la contexte du PATTEC : Les populations de tsé-tsé sont elles isolées entre bassins versants ? quelle surface de coton sur laquelle des insecticides sont utilisés suffit à isoler (durablement) des populations de tsé-tsé le long d'un réseau hydrographique donné ? Les techniques utilisées (microsatellites, morphométrie géométrique) sont-elles capables de diagnostiquer cet isolement, s'il existe ? La génétique des populations, outre son application évidente dans le cas de la lutte par SIT (Sterile Insect technique) développée en particulier par l'AIEA, est dorénavant utilisée pour répondre à des questions aussi fondamentales en matière de lutte que : peut on, ou doit on, avoir comme objectif le contrôle ou l'élimination ? La génétique des populations de tsé-tsé, qui il y a quelques années était plus que marginale, revient à l'actualité et un nombre croissant d'équipes et de bailleurs de fonds s'y intéressent, ayant compris ce que pouvaient apporter ces outils à la lutte anti tsé-tsé. Que soient remerciés ici Thierry de Meeüs, Sophie Ravel, Jean-Pierre Dujardin et Gérard Cuny, pour leur disponibilité à faire vivre et évoluer ces outils, ainsi que, auparavant, Gérard Duvallet et Dominique Cuisance qui avaient probablement « senti » tout le potentiel scientifique que l'on pouvait en tirer.

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5. Bilan et réflexions sur les activités de formation et d'encadrement de la recherche

Tout d'abord, situons le contexte dans lequel se place cette réflexion sur la formation et l'encadrement de la recherche, en tous les cas ce que j'en retiens. On ne peut en effet ignorer le cadre actuel de la réforme des Universités, des bouleversements qui touchent nos institutions de recherche, y compris celles dédiées à la recherche pour le développement (IRD, CIRAD) : UMRisation, fusion d'UR pour atteindre des effectifs importants au sein de chaque Unité, rapprochement avec les Universités, diminution des soutiens « de base » aux Unités de Recherche pour un transfert croissant des financements de la recherche vers l'Agence Nationale de la Recherche (ANR). En ce qui concerne plus particulièrement l'Institut auquel j'appartiens, les mots-clés du moment, plus particulièrement en ce qui concerne la politique de site sont « Restitution des centres IRD aux partenaires », « installation chez les partenaires », mais aussi « régionalisation », « mutualisation des moyens », et création de l'Agence Internationale de Recherche pour le Développement (AIRD), qui est supposée réunir et mutualiser l'offre Nationale de Recherche pour le Développement.
Il est évident que ces changements profonds vont avoir des conséquences importantes sur nos actions de recherche, et nos processus d'encadrement de la recherche, en particulier au Sud, mais il paraît aussi clair qu'avoir une idée précise de la nature de ces changements est à l'heure actuelle difficile, voire impossible en tous les cas pour moi.
D'un côté il est rassurant d'observer que les financements de formation à partir du niveau thèse sont de plus en plus accessibles pour les jeunes chercheurs du Sud (Département Soutien et Formation de l'IRD, financements des SCAC d'Afrique, de l'AIRD, financements sur projets d'origine diverse, européenne ou autre, publique ou privée). On les qualifiera de formation diplômante. Par opposition, des formations non diplômantes existent également, pour lesquelles nous sommes beaucoup sollicités : formations « à la carte » (CIRDES, autres instituts), formations thématiques de durée courte par les Institutions internationales (OMS/TDR, AIEA), ou formations « terrain » pour les agents de santé. Il me semblerait important, dans un souci de pérennisation et d'efficacité, de lier ces formations aux Universités, en particulier évidemment les formations de niveau 2°-3°cycle. L'exemple de la création récente du Master International d'Entomologie médicale et vétérinaire, co-délivré par les Universités de Cotonou et de Montpellier (et dont nous sommes Laboratoire d'Accueil au CIRDES), semble à suivre, non seulement car il a pour objectif d'enrayer le déclin des entomologistes médicaux et/ou vétérinaires (au moins au Nord, rapport Cuisance), mais aussi car il est garant d'une implantation durable de ces métiers, voire même d'une surveillance épidémiologique durable car réalisée par des équipes universitaires et/ou de recherche, avec un objectif de long terme et non d'urgence. A Bobo-Dioulasso avec, depuis mon arrivée, l'accroissement de l'équipe de notre UR (5 chercheurs en 2009) en accueil au CIRDES, mais aussi l'arrivée de collègues dynamiques travaillant sur l'entomologie et la génétique du paludisme, nous envisageons de créer un module de « Génétique des Parasites et des Vecteurs », qui pourrait avoir des liens privilégiés avec le MIE, mais aussi les Universités d'Abidjan, Bobo-Dioulasso, le CEMV de l'Université de Bouaké voire d'autres...

J'ajouterais un point : de septembre 2004 à avril 2008, répondant à une demande de mon Institut, j'ai accepté d'avoir la charge de la représentation IRD de Côte d'Ivoire. Ceci signifie, en addition à mon travail de chercheur, la gestion du Centre IRD d'Abidjan (15 personnels permanents, accueil permanent de 60 personnes environ), d'un centre de documentation, du parc automobile, et donc du budget de la représentation de Côte d'Ivoire, des partenariats nationaux, des conventions, des étudiants. Tout cela dans un contexte particulier qui est celui de la grosse crise franco-ivoirienne suite aux évènements d'Abidjan en novembre 2004, ce qui

a eu des conséquences importantes à mon niveau : l'IRD est toujours en situation « de veille » en Côte d'Ivoire, ce qui signifie beaucoup moins d'activités bien sûr, et une fin de la présence de chercheurs expatriés ; mais des encadrements d'étudiants sur convention et des projets de recherche qui continuent, dans un contexte bien entendu particulier. D'un point de vue de la recherche et de son encadrement, je pense que cela m'a ouvert à des domaines très différents du mien et que cela m'a beaucoup enrichi, ainsi que bien entendu sur des aspects de fonctionnement de mon Institut que je ne connaissais pas, également sur les relations avec d'autres Instituts, Universités, partenaires. Un aperçu des principales actualités de l'IRD en Côte d'Ivoire est visible sur http://www.ird.ci .

6. Présentation d'un Projet de Recherche

Démographie, Climat, et Scénarios d'évolution de la distribution des tsé-tsé et des Trypanosomoses en Afrique de l'Ouest

Introduction

Les Trypanosomoses (Trypanosomose Humaine Africaine ou THA encore appelée maladie du sommeil, Trypanosomoses Animales Africaines ou TAA) transmises par les mouches tsé-tsé, représentent toujours un problème majeur en termes de santé publique et de production animale dans les pays sub-sahariens. Elles constituent un frein au développement du bien-être des populations, à tel point que les gouvernements des pays d'Afrique regroupés au sein de l'Union Africaine ont affiché leur volonté de se débarrasser de ce problème en créant l'initiative Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Parallèlement, l'Organisation Mondiale de la Santé (OMS) vient de lancer un programme d'élimination de la maladie du sommeil (OMS, 2005).

Cependant, la question de l'évolution à long terme de ce processus se pose puisque en THA par exemple, seulement 10% des 60 millions de personnes à risque sont réellement sous surveillance, mais aussi et surtout si son support financier et technique venait à décroître comme ce fut le cas dans les années 1960 quand on pensait la THA éliminée. Pour une lutte plus efficace et pour éviter de nouvelles résurgences, nous sommes convaincus que ces processus d'élimination doivent être accompagnés par des actions de recherche notamment sur les conditions d'émergence/re-émergence/extinction de la maladie, incluant des études diachroniques. Les interactions entre le parasite (un trypanosome), le vecteur (une tsé-tsé) et l'hôte (un homme ou un animal) sont complexes et modulées par l'environnement écologique et socio-économique. La complexité du système pathogène ainsi que l'évolution rapide et instable de son environnement influencent la genèse et l'évolution de ces maladies parasitaires négligées. La lutte contre cette maladie, au-delà des connaissances sur le triptyque « parasite-vecteur-hôte » doit impérativement prendre en compte des données plus globales intégrant des facteurs climatiques, démographiques, économiques, sociaux, culturels et politiques.

En effet, la plupart des scénarios sur l'évolution climatique en Afrique prédisent une baisse de la pluviométrie et une augmentation du nombre d'accidents climatiques (Parry et al., 2007), et bien que beaucoup d'inconnues persistent (spécialement en Afrique de l'Ouest), la tendance générale serait une évolution défavorable aux tsé-tsé. Dans le même temps, l'Afrique en particulier sub-saharienne, effectue son « rattrapage démographique » : de 1950 à 2000, la population en Afrique de l'Ouest (du Sénégal au Nigéria) est passée de 64 à 240 millions d'habitants et les démographes estiment que cette population atteindra 617 millions d'habitants en 2050 (Guengant 2007). Cette augmentation des densités humaines, qui exercera une pression supplémentaire sur les biotopes des tsé-tsé devrait ainsi provoquer ainsi une réduction de leur aire de distribution. Ceci devrait donc conduire à une diminution progressive des trypanosomoses animales et humaines (Reid *et al*., 2000). Nous avons déjà pu mettre en évidence un glissement vers le sud des foyers de THA et de la présence des tsé-tsé en Afrique de l'Ouest depuis un siècle (Courtin *et al*., 2008). Nos récentes observations de terrain confirment cette tendance avec un net recul des glossines du groupe morsitans, qui par exemple au Burkina Faso ne se trouvent plus que dans certaines zones protégées (Bouyer *et al*., 2007), alors que des zones auparavant fortement prévalentes en TAA ne le sont plus (ex. Madina Diassa au Mali, V. Jamonneau com. pers. ; région d'Orodara au Burkina Faso, Z. Bengaly, com. pers.). Toutefois des observations ponctuelles permettent de penser que cette tendance verra au minimum des exceptions, voire des conditions de transmission plus sévères localement : on

peut légitimement s'inquiéter notamment de la capacité d'adaptation de *G. palpalis* aux grands centres urbains (Abidjan, Bamako, Kinshasa, Dakar) qui s'accompagnent parfois par une augmentation de la capacité de transmission (par exemple liée à des états de stress), la structuration de ses populations lui permettant de survivre à très faibles densités, et sa capacité largement sous-estimée dans la bibliographie à résister à des pluviométries très faibles (Nord Burkina Faso, Dakar…) pour peu que l'hygrométrie locale et la disponibilité de ressources alimentaires permette sa survie. Les évolutions démographique et climatique récemment publiées en Afrique de l'Ouest laissent présager une accentuation de la compétition pour les ressources entre les différentes populations, et seront probablement favorables au développement de conflits à diverses échelles. Ces conflits, par les déplacements de populations qu'ils provoquent et par leurs conséquences sur les infrastructures sanitaires et hydrauliques sont souvent synonymes d'épidémisation.

Objectifs

Ce projet se propose de créer un outil de gestion et d'aide à la décision grâce à l'élaboration de scénarios d'épidémisation/extinction des Trypanosomoses (humaine et animales) en Afrique de l'Ouest. Parvenir à cet objectif nécessite une étude multidisciplinaire incluant des données bibliographiques disponibles depuis le siècle dernier sur l'évolution du peuplement humain (augmentation des densités, migrations et déplacements, modes de vie rural/urbain) et de la répartition des bovins (densité et transhumance), ainsi que sur l'évolution des paysages (cultures, jachères, végétation « naturelle »). Ces variables seront étudiées en tenant compte des nouvelles données sur le changement climatique global, et de leur impact sur les populations de vecteurs (densité, distribution géographique et capacité vectorielle), par un travail d'investigations de terrain et d'analyses spatiales (télédétection). L'ensemble des données sera intégré dans un Système d'Information Géographique (SIG) permettant :

i. de comprendre et de mettre en évidence les facteurs prépondérants expliquant l'épidémisation/extinction des foyers de THA et des zones de TAA à l'échelle de l'Afrique de l'Ouest;
ii. d'élaborer des scénarios d'évolution des Trypanosomoses pour les années à venir afin de définir les zones prioritaires sur lesquelles cibler les efforts de lutte.

Ce travail sera développé en étroite collaboration avec les acteurs de la lutte contre la THA des pays concernés (Programme Nationaux de Lutte), des institutions nationales (Ministères des Affaires Etrangères), de recherche du sud et du nord (CIRDES, IRD, CIRAD, autres partenaires…), et internationales (OMS, FAO, PAAT, PATTEC).

Matériel & Méthodes

Approche diachonique

Une analyse bibliographique la plus complète possible sera menée sur un siècle à l'échelle de l'Afrique de l'Ouest et, portera principalement sur : l'évolution de la limite nord de répartition des tsé-tsé, l'évolution de la distribution des foyers de THA (déjà disponible), l'évolution des prévalences de TAA selon les régions, mais aussi l'évolution des densités de populations humaines et animales. Des données climatiques depuis le début du siècle seront recherchées, et nous aurons besoin d'obtenir des données sur les modèles climatiques récents prenant en compte le réchauffement global en Afrique.

Toute information « numérisable » (densités de tsé-tsé, populations) le sera afin d'effectuer des analyses diachroniques chaque fois que possible.

Des images satellites adéquates seront acquises et des analyses diachroniques environnementales seront effectuées sur des régions choisies en fonction de la présence/prévalence des trypanosomoses. En seulement quelques années certaines régions

évoluent en effet très rapidement sous l'effet de l'anthropisation (Courtin, 2008) combinée au climat.

Facteurs environnementaux des foyers de THA et des zones de TAA

Les différents modes d'occupation des terres ainsi que la végétation liée au réseau hydrographique (notamment) seront cartographiés grâce à des images satellites prises à partir du satellite LANDSAT 7 avec le capteur Enhanced Thematic Mapper Plus (ETM+) (pixel de 30m, couverture de 180*180 km) qui permettront d'établir des classifications supervisées, validées sur le terrain avec les coordonnées GPS : en particulier pour identifier et classifier les différents types de végétation le long des cours d'eau afin de définir différents types de paysage. Ces paysages aideront au diagnostic d'espaces dégradés, et/ou favorables ou défavorables à la présence des tsé-tsé, donc à la présence/absence de THA ou TAA. Les données relatives à l'occupation des terres, aux densités en hommes animaux, les paysages favorables/défavorables à la présence des vecteurs, seront superposés à l'évolution dans le temps des foyers de THA/TAA, et seront ensuite élargis à des échelles progressivement plus globales.

Il sera essentiel pour nous de pouvoir bénéficier de données climatologiques et/ou des résultats de modélisations récentes (GIEC, AMMA ou autre) ayant conduit à l'élaboration de scénarios prévisionnels d'évolution du climat dans cette région d'Afrique de l'Ouest.

De la même manière, il sera fait appel à des démographes (J.P. Guengant, IRD ; autres collaborateurs) afin de pouvoir intégrer les prévisions démographiques dans notre SIG.

Données de terrain sur la THA et les TAA

Cela fait maintenant plusieurs années que les équipes de l'IRD Unité « Trypanosomoses » collaborent totalement avec les programmes nationaux de lutte d'Afrique de l'Ouest pour la surveillance de la THA avec le soutien de l'OMS notamment. Ces résultats acquis progressivement nous donnent une légitimité et surtout une idée préliminaire sérieuse de la véritable situation de la THA en Afrique de l'Ouest (voir figure page 33) : foyers historiques, zones actuellement actives, foyers latents n'ayant pas ré-émergé ; nous pensons par exemple qu'à l'heure actuelle il n'existe plus de THA endémique au nord de l'isohyète 1200 mm (en Afrique de l'Ouest). Mais l'on peut également observer que beaucoup de régions restent totalement inconnues de nos jours du point de vue de la THA, car non prospectées depuis plusieurs années (zones forestières de Guinée, Liberia, Sierra Leone, par exemple). Afin de compléter cette étude diachronique et d'actualiser la situation de la THA, des prospections médicales devront donc être organisées dans ces régions prioritairement.

Ces prospections médicales seront systématiquement organisées avec le PNLTHA du pays concerné. La procédure utilisée sera celle recommandée par l'OMS 1998 : une visite aussi exhaustive que possible de la population à risque dans tous les villages et hameaux de la zone, un poste d'enregistrement, un ou plusieurs postes de prélèvement sanguin au bout du doigt, un premier test sérologique de masse, le CATT (Card Agglutination Test for Trypanosomiasis, Magnus *et al*., 1978), pour sélectionner des « suspects sérologiques » sur lequel ce CATT est refait sur plasma pour améliorer la spécificité. Les tests parasitologiques sont ensuite faits sur les séropositifs, soit sur le sang (mini Anion Exchange Column technique, mAECt), soit un examen direct du suc des ganglions lymphatiques en cas de présence d'adénopathie. Les malades seront convoqués dans un centre de traitement où l'examen du liquide céphalo-rachidien permettra de déterminer la phase de la maladie qui conduit à choisir le traitement approprié, qui sera mis en œuvre par le PNLTHA.

En terme de TAA, une actualisation des prévalences est nécessaire, et nous bénéficierons des données du PATTEC qui devront être complétées par des données hors zone PATTEC, par exemple par le CIRDES.

Résultats attendus

La combinaison des facteurs biotiques et abiotiques, leur présence/absence, à travers un SIG le plus complet possible devra permettre d'expliquer la présence/absence des Trypanosomoses africaines (humaine et animales), leur évolution diachronique afin de proposer des scénarios basés sur l'épidémisation/extinction qui serviront d'aide à la décision pour prioriser les zones d'intervention médicale et de lutte anti-vectorielle. Ce type d'étude prédictive devrait ensuite pouvoir s'appliquer aux Trypanosomoses dans d'autres zones (Afrique Centrale et Afrique de l'Est), voire être étendu à d'autres pathologies transmises par des vecteurs. Le produit final facilitera l'aide à la décision et contribuera à réduire les coûts de la surveillance à l'échelle de l'Afrique de l'Ouest en ciblant précisément les zones prioritaires en collaboration étroite avec le PATTEC (Projet d'élimination des tsé-tsé en Afrique de l'Ouest financé par la Banque Africaine de Développement). De plus, en identifiant les principaux facteurs de risque et de « non risque », il contribuera à l'élaboration des stratégies de lutte.

Il semble d'ores et déjà intéressant d'étendre cette approche à d'autres maladies à vecteurs que les Trypanosomoses.

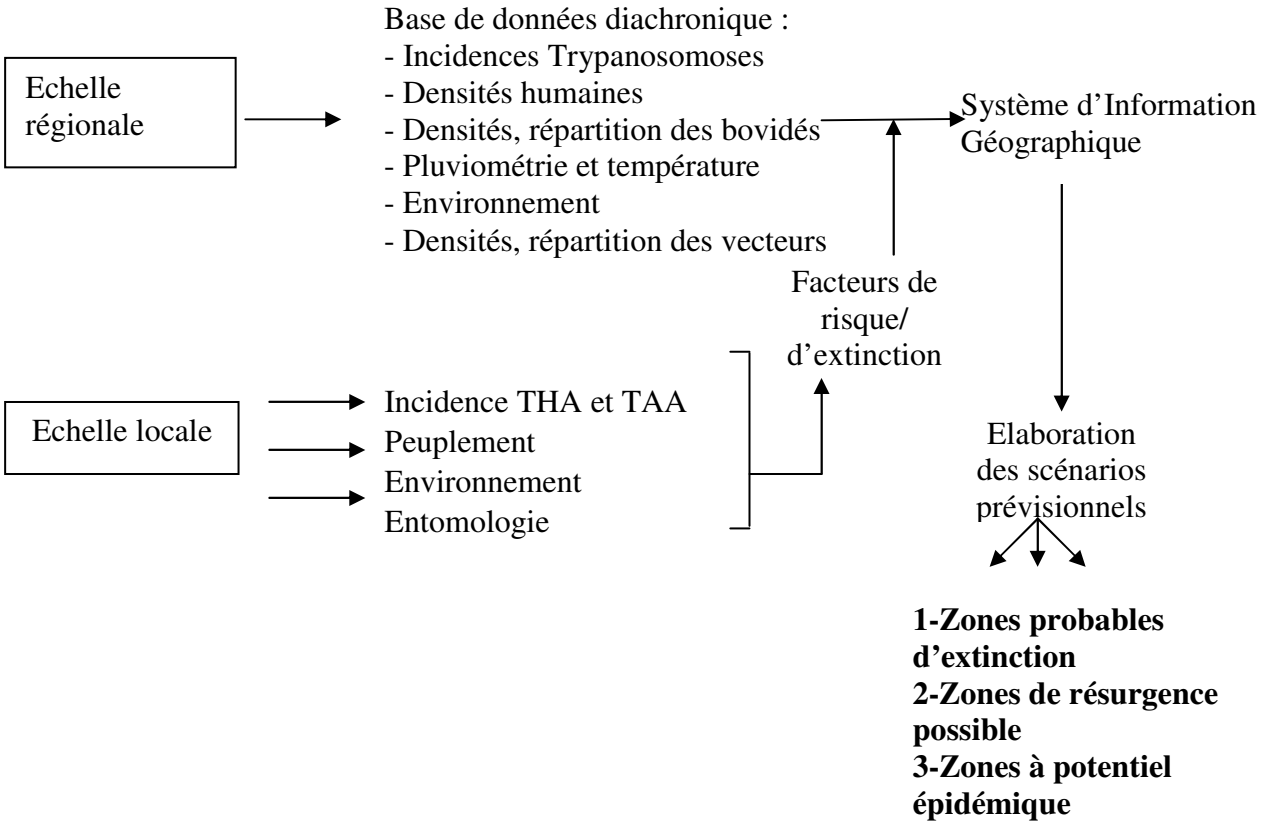
Considérations éthiques

Seuls seront inclus dans cette étude les individus qui auront été informés du protocole dans leur langue locale, et auront signé un accord de consentement. Les malades seront traités par le Programme National de Lutte contre la THA de leur pays selon les recommandations existantes. Le protocole sera soumis au comité d'éthique du pays concerné et de l'Institut coordonnant le projet (IRD, CIRDES).

Faisabilité

Le contexte semble idéal pour mener à bien ce type de projet au vu de ces éléments simultanés : les rapports récents du GIEC, les prévisions à peine publiées des démographes sur l'Afrique d'ici 2050, le démarrage maintenant concret du PATTEC en Afrique de l'Ouest, et des projets récemment financés que nos équipes et partenaires avons obtenus au CIRDES sur les Trypanosomoses. Le projet sera exécuté principalement au CIRDES, Institut de Recherche basé au Burkina Faso qui a un mandat régional puisque son Conseil d'Administration est composé des pays signataires du "Conseil de l'Entente". La synergie de collaboration avec le PATTEC sera totalement favorisée puisque le coordonnateur PATTEC (Dr I. Sidibé) est également basé à Bobo-Dioulasso, et nous avons de longues années de fructueuse collaboration. Au CIRDES sont basés plusieurs chercheurs de l'UMR IRD-CIRAD 177 (parasitologie, vecteurs, interactions du système pathogène), un géographe ainsi que les chercheurs du Sud et le post-doctorant. Ceci assurera une coordination très opérationnelle puisque ces équipes ont une longue tradition de travail en commun. D'éminents chercheurs en démographie sont également basés au Burkina Faso (J.P. Guengant, représentant IRD au Burkina Faso), et des réunions préliminaires sont déjà programmées : 24 au 27/11/2008, Conférence internationale organisée par le CIRDES sur « Evolution démographique et climatique : impacts sur les maladies à transmission vectorielle en Afrique de l'Ouest ». Un étudiant burkinabé (J. ROUAMBA, Univ. Montpellier 3) vient de commencer une thèse sur : « Dynamique de peuplement en Afrique de l'Ouest dans un contexte de variabilité démo-climatique : impact sur la distribution spatiale des glossines et des trypanosomoses. »

Organisation du projet



Sources de financement possibles

ANR, AIRD

Une partie de ces financements est déjà acquise :

- Union Européenne INCO-DEV pour la structure des tsé-tsé (2006-2009)
- Projet FSP/MAE « REFS » et OMS pour les prospections médicales THA (2007-2009) et des opérations de lutte anti-vectorielle
- PATTEC pour les données présence/absence des tsé-tsé dans les zones d'étude transfrontalières au Burkina Faso, au Mali, au Ghana.

7. Choix de publications représentatives jointes au document

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* Lefrançois T., Solano P., De la Rocque S., Bengaly Z., Reifenberg J.M., Kabore I. & Cuisance D. (1998). New epidemiological features on animal trypanosomosis by molecular analysis in the pastoral zone of Sideradougou, Burkina Faso. *Molecular Ecology*, 7, 897-904.

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ACTA TROPICA

Microsatellite markers for genetic population studies in *Glossina palpalis* (*Diptera*: *Glossinidae*)

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Abstract

Little is known about tsetse intraspecific variability and its consequences on vectorial capacity. Since isoenzyme analyses revealed little polymorphism, microsatellite markers have been developed for *Glossina palpalis gambiensis* species. Three loci have been identified and showed size polymorphisms for insectarium samples. Moreover, amplifications were observed in different species belonging to *palpalis* group. These molecular markers will be useful to estimate gene flow within *G. p. gambiensis* populations and analyses could be extended to related species. © 1997 Elsevier Science B.V.

Keywords: Microsatellite; *Glossina palpalis*; Population genetics; Trypanosomosis; *G. fuscipes*; *G. morsitans*

1. Introduction

Most species of the genus *Glossina* play a potential vector role in the transmission of African *Trypanosomosis*, which has considerable economic impact in subsaharan

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Africa (Janhke et al., 1988). In West Africa, species of the *palpalis* group (subgenus *Nemorhina*) are involved in transmission of Animal trypanosomosis (nagana) and Human trypanosomosis (sleeping sickness). Despite their importance little is known about tsetse population genetics and their imp]ications for the transmission of trypanosomes: very little information is available on the possible structuration of tsetse populations which could lead them to express resistance to control measures, by avoiding traps or treated animals for example. Intraspecific variation and related differential vectorial capacity is suspected to occur in natural *G. palpalis gambiensis* populations (Bauer et al., 1995; Solano et al., 1996). However, previous studies using isozyme analyses were undertaken only for interspecies comparisons (Gooding et al., 1991) or for purpose of genetic assignment of loci (Gooding and Rolseth, 1995). Natural populations of tsetse flies of Burkina Faso showed little polymorphism using isozyme data on five loci (Gooding, 1981). Genetic studies were hence of limited value because of the lack of accurate technologies.

Among insects, microsatellite loci have mostly been developed for social species like ants (Gertsch et al., 1995), bees (Estoup et al., 1993, 1995), or wasps (Hughes and Queller, 1993). In the field of medical or veterinary entomology, studies are still rare, and microsatelllites have only been developped in *Anopheles gambiae* (Zheng et al., 1993, 1996; Lanzaro et al., 1995) and *Simulium damnosum* (Dumas, unpublished results).

This paper reports on the isolation of microsatellite sequences in *G. p. gambiensis*, a riverine species widespread in West Africa and their potential use for population genetics in this taxa and in related species.

2. Material and methods

DNA (20 μg) from 50 individual *G. p. gambiensis*, originating from the CIRAD/ORSTOM insectarium (Montpellier, France), was digested to completion overnight with *Hae*III. The 400- 800 bp fraction was recovered and ligated into the dephosphorylated EcoRV site of M13 BM 20 (Boehringer–Mannheim). Ligation products were used to transform *E.coli* XLl Blue cells and 4500 recombinants clones were lifted on Hybond-N^+ membranes. Hybridizations were carried out with (CA)n and (GA)n probes, labelled with dCTP-$\alpha[^{32}P]$, using rapid hybridization buffer (Amersham) according to manufacturer instructions. Positive clones were dot-blotted and re-screened to ensure specificity. Eleven clones were kept after this secondary screening. Eight of them were sequenced by the dideoxy-chain termination method, using the Taq dye primer kit and an automatic sequencer (Applied Biosystems).

Template DNA for PCR was prepared by incubating two legs of a fly in 5% chelex for 1 h at 56°C, then 30 min at 95°C. Amplification reactions were performed in a Perkin Elmer thermal cycler, in a final volume of 50 μl containing as final concentrations 1 × Appligene incubation buffer with 1.5 mM $MgCl_2$, 200 μM of each dNTP, 15 pmol of each primer and 0.5 U Appligene Taq Polymerase. Samples were first denatured during 90 s at 92°C and then processed through 35 cycles consisting of 30 s at 92°C, 30 s at 50°C for loci 55.3 and 19.62 and 48°C for

locus 69.22 and 1 min at 72°C. The last elongation step was lengthened to 10 min. An amount of 15 μl of each amplified sample was resolved on 12% non-denaturing polyacrylamide gel.

3. Results

Of these eight clones sequenced, four were false positives and microsatellite sequences were successfully obtained for four clones. The presence of false positives can be explained by an imperfect homology of sequences between the clones and the microsatellite probes due to the low stringency of the hybridization washes. Two clones had microsatellite sequences located too close to the cloning site to allow primer selection; fortunately, as one of them (19.62) owned a (TA) repeat also, three pairs of primers could be designed.

A sequence was considered as a microsatellite if the number of repetitions of the dinucleotide motif was six at a minimum (Stallings et al., 1991). According to Weber (1990), the three microsatellite loci were classified as 'perfect' for 69.22 and 19.62 ($(TA)_{10}$ and $(GT)_{12}$, respectively) and 'imperfect' for 55.3 $(GT)_{14}$ $GC(GT)_4$.

Eight individual *G. p. gambiensis* from the insectarium were individually tested by PCR with the three primer pairs and PCR products were size-fractioned on 12% acrylamide gels with appropriate markers. Results were as follows: locus 55.3 showed four alleles, locus 19.62 showed three and locus 69.22 showed two alleles (Table 1). Allele size was highly variable; for example, 20 bp separated the largest and smallest alleles at locus 19.62 (Table 1).

The three primer pairs gave also a strong signal with wild *G. p. gambiensis* from Mali; the three individuals tested had some alleles in common with some of the insectarium tsetse, for example allele 176 bp at locus 19.62, which appears as the most common. Wild *G. palpalis palpalis* from Cameroun and a laboratory colony of *G. fuscipes fuscipes* gave also scorable signals for the three primer pairs with intra-colony variability at the three loci for *G. p. palpalis*, and at loci 55.3 and 19.62 for *G. f. fuscipes* (Table 2). Only primer pairs 19.62 amplified an appropriate sized product from DNA of *G. tachinoides*. No amplification signal could be obtained at

Table 1
Characteristics of the three microsatellite loci among a laboratory sample of eight *G. p. gambiensis*

Locus	Repeat sequence	Allele sizes (bp)	Primer sequence
55.3	$(GT)_{14}(GC)$ $(GT)_4$	181, 183, 187, 197	5′GTACTCAACGTGGTGCTTAAAGTTG3′
			5′GTCTGAGATAGGACCATTTATCG3′
19.62	$(GT)_{12}$	176, 178, 196	5′CAGATATGCTACACTTGGTCAGC3′
			5′GCATTTAATGTTATACACTGAAGG3′
69.22	$(TA)_{10}$	198, 200	5′CAAAACTCGACCAAATTGACCG3′
			5′CGATAATGATACGATTAAATCAAACC3′

Table 2
Size of the bands (when observed) in other tsetse taxa

Locus	Bands observed (bp)				
	G. p. palpalis	*G. f. fuscipes*	*G. tachinoides*	*G. m. submorsitans*	*G. m. morsitans*
55.3	175, 171	181, 185	None	None	None
19.62	170, 174	174, 182	None	None	None
69.22	194, 200	192	None	None	None

any locus with either *G. morsitans morsitans* nor than with *G. morsitans submorsitans*.

4. Discussion

The (CA)n and (GA)n probes used in this work allowed three primer pairs which showed size polymorphisms in a laboratory sample of *G. p. gambiensis*, to be designed. The fact that one locus (69.22) consisted of a (TA) repeat whereas the probes used (CA)n and (GA)n could be explained by the presence of a second GT repeat too close to the cloning site to allow primer selection.

The tsetse individuals from different origins used in this study were just tested for scorable amplifications. After this first step, heritability of the presumed alleles should be demonstrated, then the microsatellite markers will be used to estimate gene flows within species. *G. p. gambiensis* is of particular interest since previous work has shown great plasticity of behaviour in Burkina Faso (Challier, 1973; Bauer et al., 1995; Solano et al., 1996) and great variability in transmitting *Trypanosoma brucei gambiense* (Elsen et al., 1994). At the moment, there is no strong evidence that tsetse populations are structured, as is the case in *Anopheles gambiae*, for example (Lanzaro et al., 1995), but this could be due to the lack of studies of this type of tsetse.

All tsetse belong to one genus *Glossina*, that is divided into three subgenera, *Austenina* (*fusca* group), *Nemorhina* (*palpalis* group) and *Glossina* (*morsitans* group). *G. m. submorsitans* and *G. m. morsitans* are in the *morsitans* group and could not be amplified with any primer sets used in this study. In contrast, all three loci were correctly amplified in both *G. p. palpalis* and *G. f. fuscipes* from the *palpalis* group. Indeed these species were first classified as a single one, *G. palpalis* (Van der Planck, 1949), and they showed great similarity at isoenzyme loci (Fig. 1). Finally, *G. tachinoides* belongs also to the *palpalis* group, but is known to be quite different from *G. palpalis*, regarding ecological behaviour as well as genetic data (Gooding et al., 1991). In this work, the primer specificity reflected well the generally accepted phylogenetic relationships between the tsetse taxa (Fig. 1). In the future, these genetic studies could be extended to related species because interspecific conservation of flanking sequences will support use of these loci.

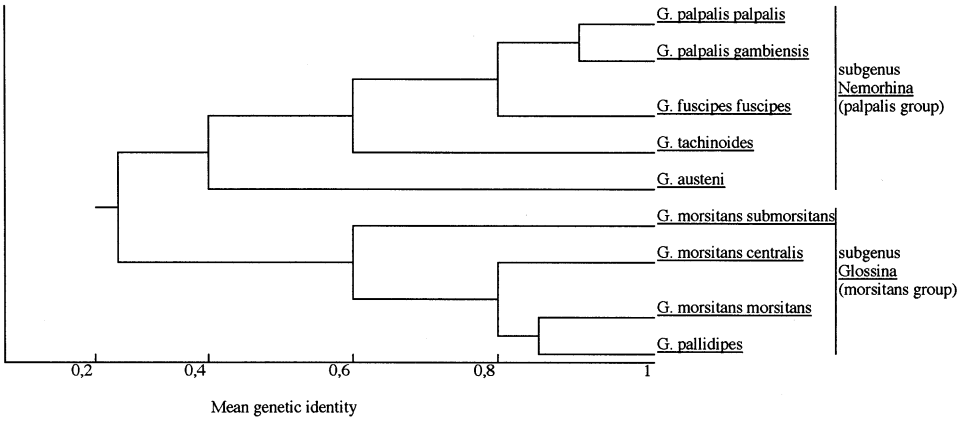

Fig. 1. Phenogram for nine taxa of tsetse flies, based upon loci for 12 enzymes (Gooding, 1981, modified).

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New epidemiological features on animal trypanosomiasis by molecular analysis in the pastoral zone of Sideradougou, Burkina Faso

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Abstract

A multidisciplinary work was undertaken in the agropastoral zone of Sidéradougou, Burkina Faso to try to elucidate the key factors determining the presence of tsetse flies. In this study the PCR was used to characterize trypanosomes infecting the vector (*Glossina tachinoides* and *Glossina palpalis gambiensis*) and the host, i.e. cattle. A 2-year survey involved dissecting 2211 tsetse of the two *Glossina* species. A total of 298 parasitologically infected tsetse were analysed by PCR. *Trypanosoma vivax* was the most frequently identified trypanosome followed by the savannah type of *T. congolense* and, to a lesser extent, the riverine forest type of *T. congolense*, and by *T. brucei*. No cases of *T. simiae* were found. From the 107 identified infections in cattle, the taxa were the same, but *T. congolense* savannah type was more frequent, whereas *T. vivax* and *T. congolense* riverine forest types were found less frequently. A correlation was found between midgut infection rates of tsetse, nonidentified infections and reptile bloodmeals. These rates were higher in *G.p. gambiensis*, and in the western part of the study area. *T. vivax* infections were related to cattle bloodmeals, and were more frequent in *G. tachinoides* and in the eastern study area. The PCR results combined with bloodmeal analysis helped us to establish the relationships between the vector and the host, to assess the trypanosome challenge in the two parts of the area, to elucidate the differences between the two types of *T. congolense*, and to suspect that most midgut infections were originating from reptilian trypanosomes.

Keywords: African trypanosomes, Burkina Faso, cattle, *Glossina palpalis gambiensis*, *Glossina tachinoides*, PCR

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Introduction

Animal trypanosomiasis represents a major constraint in Subsaharan Africa, and it is considered the most important vector-borne cattle disease transmitted by vectors in this region (FAO 1994). Nonpolluting control methods are now currently used and can prove successful under certain conditions (Vale *et al*. 1988; Cuisance *et al*. 1991; Bauer *et al*. 1992, 1995). However, these methods require a thorough knowledge of the epidemiological situation, which is both time and labour consuming. Also, there is still a great lack of knowledge about the diagnosis of the disease, the characterization of the pathogenic agents, and the complex relationships between the tsetse and the parasites they transmit (Welburn & Maudlin 1989; Maudlin 1991; Desquesnes & de La Rocque 1995; Solano *et al*. 1996; Van den Abbeele *et al*. 1996; Reifenberg *et al*. 1997a). The PCR on tsetse organs using specific primers for different species or subgroups of trypanosomes has already proven successful in characterizing trypanosomes at the molecular level and in revealing multiple infections in single tsetse from the field (Masiga *et al*. 1992; Solano *et al*. 1995; McNamara *et al*. 1995; Woolhouse *et al*. 1996; Reifenberg *et al*. 1996). PCR on buffy-coat samples seems a promising tool (Majiwa *et al*. 1994; Penchenier *et al*. 1996; Desquesnes & Tresse 1996).

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In the pastoral zone of Sideradougou, southwest of Burkina Faso, a multidisciplinary work tried to identify the key factors determining the presence of tsetse flies (De La Rocque & Cuisance 1997). All data on entomology, parasitology, livestock, drainage lines, environment, and human pressures were sampled in order to be included in a Geographic Information System. A tsetse eradication program had already been conducted 13 years earlier in this area through the combined use of impregnated screens and the sterile insect technique (Cuisance *et al*. 1984; Politzar & Cuisance 1984; Bauer *et al*. 1988).

The present study reports on one of the aspects of this program, i.e. the PCR characterization of the trypanosomes infecting *Glossina tachinoides* and *Glossina palpalis gambiensis*, two widespread riverine species of the *palpalis* group in West Africa, as well as cattle in the same area.

Materials and methods

Entomological surveys

The study area lies in the south sudanian savannah zone (4°20′ west, 10°55′ north); the hydrographic network is principally represented by the Koba and the Tolé rivers (Fig. 1). A total area of 120 km was prospected in 1996 and 1997, with biconical (Challier & Laveissière 1973) and Vavoua (Laveissière & Grebaut 1990) traps deployed at 100-m intervals (De La Rocque & Cuisance 1997). Dissections were carried out on nonteneral flies. The dissecting instruments were cleaned between each dissected organ and each dissected fly with sodium hypochloride followed by rinsing with sterile distilled water. The proboscis was first pulled out from the fly, then the salivary glands and the midgut. The three organs from each fly were then examined for trypanosomes under a microscope at 400× magnification. When a tsetse organ was found to be positive it was placed in a single Eppendorf tube containing 50 µL of sterile distilled water (except the proboscis which was put in a tube without water).

Epidemiological surveys

In 1996 and 1997, sentinel herds were regularly checked for trypanosome infections using the buffy-coat/dark ground method (Murray *et al*. 1977) and thin blood smear. A measure of the haematocrit was routinely performed (Z. Bengaly *et al*. unpublished). Furthermore, cattle of farmers living close to the Koba and the Tolé rivers who complained about the health of their animals were also examined.

A second buffy-coat sample was collected when animals were found positive or for every individual with a haematocrit value below 25%. The sample was immediatly emptied into an Eppendorf microtube containing 50 µL of sterile, distilled water, conserved at the surrounding temperatures and brought back to the CIRDES laboratory.

Preparation of samples for PCR

Sample preparation was performed according to Penchenier *et al*. (1996). Each tsetse organ was crushed with clean instruments. Salivary glands were used as crude template for PCR after simple agitation. The midgut, proboscis, and buffy coat were centrifuged at 13 000 *g* for 8 min. After removing 30 µL of the supernatant, an amount of 50 µL of Readyamp (Promega) was added to the pellet. After agitation the samples were incubated for 20 min at 56 °C, then at 95 °C for 8 min. They

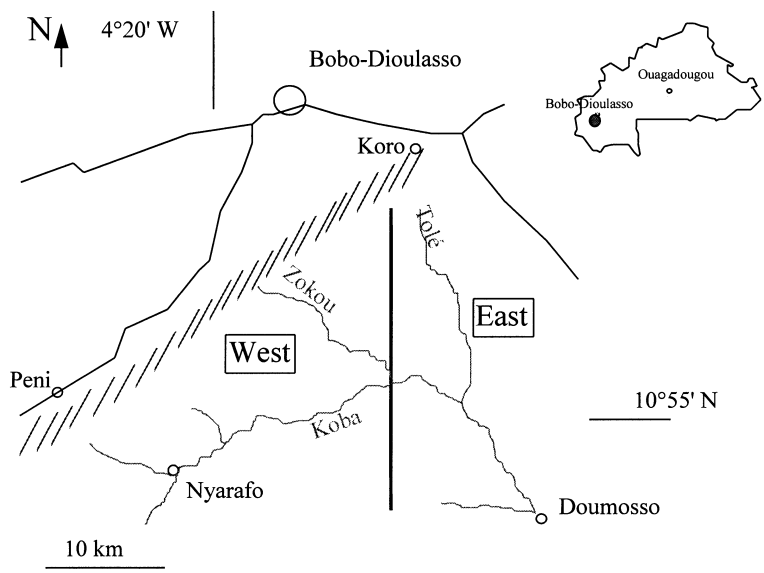

Fig. 1 Map of the area under study. The map shows the prospected gallery along the Koba and Tolé rivers, main road (–), and escarpment (indicated by diagonal lines). The vertical bar separates western and eastern parts of the area. The small map of the Burkina Faso indicates the localization of the Sidéradougou area (near Bobo-Dioulasso).

were centrifuged at 13 000 *g* for 2 min and then frozen at – 20 °C before further handling.

PCR analysis

Oligonucleotide primers for trypanosome characterization were kindly provided by CIRAD–EMVT Baillarguet, France, and ILRI, Nairobi, Kenya. These primers amplified repetitive nuclear DNA sequences specific of the following taxonomic groups: *Trypanosoma* (*Nannomonas*) *congolense* (*T.c.*) savannah type (Majiwa *et al*. 1993) and riverine forest type (Masiga *et al*. 1992), *T.* (*Nannomonas*) *simiae* (Masiga *et al*. 1992), *T.* (*Duttonella*) *vivax* (Dickin & Gibson 1989), and *T.* (*Trypanozoon*) *brucei sensu lato* (Moser *et al*. 1989).

Standard PCR amplifications and electrophoresis were carried out as described previously (Solano *et al*. 1995). Parasitologically positive samples which gave negative results were systematically subjected to a new amplification reaction for 35 or 40 cycles and, if still negative, were DNA-extracted and submitted again to an amplification reaction.

Each amplification was performed in duplicates to ensure reproducibility, and a positive control (with reference DNA of each type of trypanosome) and a negative control (without DNA) were added in each PCR assay.

When the amplification reaction gave a signal of the expected size of DNA with the primers used without signal in the negative control, the trypanosome was considered as characterized.

Analysis of tsetse blood meals

When the midgut of the flies contained sufficient blood residues, it was crushed on Whatman paper no. 1, air-dried and brought back to CIRDES for analyses. An ELISA test of antisera of defined host species was used (Kaboré *et al*. 1994).

Statistical tests

Proportions were statistically analysed with a classical $\chi 2$ test, using Yates correction for small numbers (< 5 by cell), and means were compared using a Student's *t*-test.

Results

Parasitological results

Out of 4357 captured flies (57% *Glossina tachinoides*, and 43% *Glossina palpalis gambiensis*), 1210 *G. tachinoides* and 1001 *G.p. gambiensis* were dissected.

Among the dissected flies the overall infection rates were not statistically different between *G. tachinoides* (14.6%) and *G.p. gambiensis* (12.1%).

Infections of both proboscis and midgut were not statistically different between *G. tachinoides* (13.6%) and *G.p. gambiensis* (7.4%). Infections of midgut alone were found less frequently in *G. tachinoides* than in *G.p. gambiensis* ($P < 0.001$). In contrast, proboscis infections were more abundant in *G. tachinoides* than in *G.p. gambiensis* ($P < 0.001$) (Table 1).

PCR analysis

A total of 298 infected tsetse (177 *G. tachinoides* and 121 *G.p. gambiensis*) were analysed by PCR. The PCR identification rate of trypanosomes in the flies was higher in *G. tachinoides* than in *G.p. gambiensis* ($P < 0.001$; Tables 1 and 2).

Combining the two species, the PCR identification rate of trypanosomes was lower for midgut infections only (15.8%) than for infections of both proboscis and midgut (72.7%) and proboscis alone (87.9%), $P < 0.001$.

In both tsetse species, *Trypanosoma vivax* was dominant when looking at overall infections. The tsetse were also infected by the savannah and the riverine-forest types of *T. congolense* and by *T. brucei*. *T. simiae* was never detected. *T. c.* savannah type, *T. vivax*, and *T. brucei* infection rates were significantly higher in *G. tachinoides* than in *G.p. gambiensis* (Table 2).

When comparing the infections in *G. tachinoides* and *G.p. gambiensis*, mature infections (defined as proboscis or salivary glands with PCR-identified trypanosomes) were more frequent in the first (Table 2). The savannah and riverine-forest types of *T. congolense* and *T. vivax* mature infection rates were significantly higher in *G. tachinoides* than in *G.p. gambiensis*. For example, in *G. tachinoides*, 85.7% of the *T.c.* riverine forest-type infections were mature; none of the *T.c.* riverine forest-type infections of *G.p. gambiensis* were mature ($P < 0.01$) (Table 2).

Comparison between parasitological and PCR results

Looking at overall infections of both proboscis and midgut (parasitologically classified as *Nannomonas*), 54.5% were effectively attributed by PCR to *Nannomonas* (with 21.2% of mixed infections with another trypanosome), 15.2% to

Table 1 Epidemiological differences between *Glossina tachinoides* and *G. palpalis gambiensis*

	G. tachinoides		*G.p. gambiensis*
Midgut infections	39.0%	***	68.6%
Nonidentified trypanosomes	36.2%	***	71.1%
Reptile bloodmeal	15.4%	***	34.5%
Proboscis infections	45.8%	***	21.5%
Trypanosoma vivax	46.9%	***	18.2%
Ruminant bloodmeal	46.3%	***	19.0%

Significant differences: ***, $P < 0.001$. The midgut infections and proboscis infections refer to microscopic examination; the non-identified trypanosomes and *T. vivax* are PCR results.

Table 2 PCR results of infected flies

	Overall infections			Mature infections		
	G. tachinoides		*G.p. gambiensis*	*G. tachinoides*		*G.p. gambiensis*
Identified infections	63.8%	***	28.9%	54.2%	***	23.1%
T.c. savannah type	20.3%	**	8.3%	12.4%	*	4.1%
T.c. riverine-forest type	7.9%		4.1%	6.8%	**	0%
T. vivax	46.9%	***	18.2%	46.3%	***	18.2%
T. brucei	9.6%	*	2.5%	5.1%		2.5%

Percentages of identified infections and mature infections by PCR among infected flies. The column 'overall infections' refers to positive PCR results obtained on any tsetse organ. A 'mature infection' is a positive PCR result obtained in the mouthparts and/or the salivary glands of the tsetse. The sum of the different percentages is more than 100% because of mixed infections. Significant differences: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

T. vivax, 3% to mixed *T. vivax*/*T. brucei*, and 27.3% to other nonidentified trypanosomes. From the proboscis infections (parasitologically interpreted as *Duttonella*), 86% were identified by PCR as *T. vivax*.

The PCR allowed characterization of 15.8% infections of midgut alone which could not have been identified by classical criteria. Among them the highest infection rate was with *T.c.* savannah type (11.2%) vs. *T.c.* riverine forest type (3.3%) and *T. brucei* (3.9%).

Bloodmeal analyses

A total of 233 blood relics found in the midgut gave interpretable results. The bloodmeal origins differed significantly between the two tsetse species. Bloodmeals from ruminants were more frequent in *G. tachinoides* than in *G.p. gambiensis*. In contrast, bloodmeals from reptiles were more frequent in *G.p. gambiensis* (see Table 1). Most of the other bloodmeals originated from suids, and from humans, dogs and equines in lower proportions.

Spatial analysis

As a first step in the spatialization of the results, differences were observed between the western and eastern parts of the Sidéradougou zone (see Table 3).

Localization of the tsetse showed that 52% of the *G. tachinoides* and 60% of the *G.p. gambiensis* were captured in the western area. The infection rates were higher in the western than in the eastern area for *G. tachinoides* (19% vs. 11.8%, $P < 0.01$) and for *G.p. gambiensis* (17.3% vs. 2.3%, $P < 0.001$).

For the two combined species, midgut infections and bloodmeals from reptiles were more frequent in the western than in the eastern area, whereas proboscis infections and bloodmeals from ruminants were more frequent in the eastern.

The PCR identification rate of trypanosomes in the flies was higher in the eastern than in the western area, and *T. vivax* infection rate was higher in the eastern area.

Buffy-coat analyses by PCR

Buffy-coat analysis was performed by PCR on 137 cattle found infected by parasitological analysis, or with a haematocrit lower than 25%. The PCR made it possible to characterize 90% of parasitologically positive animals. Furthermore, 50% of parasitologically negative cattle with a haematocrit below 25% were found to be infected by PCR analysis.

Among infections identified by PCR (see Table 4), *T. congolense* infections (64.3%) were more frequent than *T. vivax*

Table 3 Epidemiological differences between western and eastern areas

	West		East
Parasitological infection rate	23%	***	10.0%
Proportion of midgut infections	65.5%	***	20.0%
Proportion of proboscis infections	22.7%	***	64.2%
Proportion of *T. vivax* by PCR	21.2%	***	65.3%
Proportion of bloodmeals on ruminants	21.2%	**	42.8%
Proportion of non identified infections by PCR	66.5%	***	15.8%
Proportion of bloodmeals on reptiles	36.4%	**	16.9%

Significant differences: **, $P < 0.01$; ***, $P < 0.001$.

Table 4 PCR analysis of tsetse and cattle infections

	Tsetse		Cattle
Identified by PCR	124		107
T.c. savannah type	21.8%	***	64.3%
T.c. riverine forest type	9.7%	*	3.1%
T. vivax	83.9%	***	39.8%
T. brucei	9.7%		11.2%
Mixed	20.2%		16.3%

Percentages of identified infections by PCR for the combined two tsetse species (mature infections) and for the buffy-coat analysis of cattle blood. The sum of the different percentages is more than 100% because of mixed infections. Significant differences: *, $P < 0.05$; ***, $P < 0.001$.

(39.8%, $P < 0.001$) which again were superior to *T. brucei* (11.2%, $P < 0.01$). The savannah type of *T. congolense* was much more frequent than the riverine forest type ($P < 0.001$). Mixed infections involving various trypanosome groups were observed (see Table 4). The *T.c.* riverine forest type was found only in three suspect animals (parasitologically negative, with haematocrit under 25%).

Discussion

In this work, the trypanosome infection rates of the two tsetse taxa were higher than generally reported in West Africa (Nash & Page 1953; Jordan 1961; Baldry 1964). This was notably true when looking at mature infection rates in *Glossina palpalis gambiensis* in West Africa (Squire 1954), particularly in Burkina Faso (Bauer *et al*. 1995; Solano *et al*. 1996), but also to a lesser extent for *G. tachinoides* (see Buxton 1955).

When parasitological interpretation was compared with PCR looking at the two tsetse species, 45.5% of the presumed (i.e. by microscopic examination) *Nannomonas* infections were attributed by PCR as *Trypanosoma vivax*, *T. brucei* or were not identified. These infections were actually characterized mainly as *T. vivax* in the proboscis with a concomitant infection of another species in the midgut, thus leading to a false diagnosis of *Nannomonas*.

The primer sets used in this work allowed the distinction of *T. congolense* and *T. simiae*. However, in our study, *T. simiae* was never characterized by PCR, although it had already been found in these tsetse taxa in other parts of Burkina Faso (Solano *et al*. 1996; Reifenberg *et al*. 1997b). Trypanosomes at the infraspecies taxonomic level could also be differentiated (savannah and riverine forest types of *T. congolense*), which might infect different host species (Sidibe 1996; Reifenberg *et al*. 1997b). The PCR technology also made it possible to detect mixed infections involving two types of trypanosomes, and to characterize some of the midgut infections.

The analysis of the buffy coat using PCR proved a very effective diagnostic tool in this work because 90% of parasitologically infected and 50% of suspect cattle were infected. Majiwa *et al*. (1994), also reported positive PCR results on 12.5% antigenemic but aparasitaemic cattle. The results also underline the value of haematocrit as an indicator of cattle herd health. Combining parasitological prevalence analyses with haematocrit readings provides a reliable method to assess the trypanosome challenge.

The identification rate of infections in the midgut alone of the tsetse was very low. This could have been due to technical failure (PCR inhibitors in the midgut) or to the fact that the majority of trypanosomes in the midgut were eventually found to be *T. grayi*- or *varani*-like (Hoare 1931; Minter-Goedbloed *et al*. 1993) infections, not recognized by our set of primers. DNA extraction, known to prevent PCR inhibitors, failed to characterize the nonidentified midgut infections. Second, in some of these midguts, the trypanosomes were observed just at the limit between the midgut and the hindgut, suggesting the presence of reptile-specific trypanosomes. This hypothesis was strengthened by the various reptile fauna (crocodiles, monitor lizards) frequently seen in the area, on which tsetse of the *palpalis* group are known to feed preferentially (Jordan 1961; Gouteux *et al*. 1982; Laveissière & Boreham 1982). This also appeared to be true for *G.p. gambiensis* in Burkina Faso (Bauer *et al*. 1995). In this study, the overall negative PCR results on observed infections were higher in *G.p. gambiensis* than in *G. tachinoides*, the first species having a higher proportion of infections in the midgut alone, with a higher proportion of reptile bloodmeals. Furthermore, 75% of bloodmeals originated from reptiles in nonidentified midgut infections. Therefore, it is postulated that most of these midgut infections can be attibuted to *T. grayi*- and *T. varani*-like trypanosomes. However, the other infected midguts which were not characterized could also harbour other pathogenic trypanosomes not recognized by the set of primers used in this work (Masiga *et al*. 1992; Majiwa *et al*. 1993; McNamara *et al*. 1994).

When looking at overall infections characterized by PCR in both species, *T. vivax* was the most frequent. This was not the case in the examined cattle, as the most frequent identified trypanosome was *T.c.* savannah type. All infected tsetse with a bloodmeal of ruminant origin were infected by *T. vivax*, confirming the correlation between the acquisition of *T. vivax* infections and host preferences (Jordan 1965; Ryan *et al*. 1986). *T. vivax* infection rates were higher in *G. tachinoides* than in *G.p. gambiensis*; this was related to a higher proportion of infections in the proboscis alone and with a higher proportion of ruminant bloodmeals.

These results emphasize the interest of using PCR and systematic bloodmeal analysis of infected tsetse, eventually improving the latter through the use of PCR methods.

The discrepancies between the dominant trypanosome taxa in the tsetse (*T. vivax*) and in cattle (*T.c.* savannah) raise the followings questions. (i) Did we catch the true vectors, i.e. are the tsetse entering the traps those that transmit the trypanosomes found in cattle? If some vector populations could avoid the traps, this could account for some of the observed discrepancies (RTTCP 1996). (ii) Were the cattle of this area able to control *T. vivax* infections? (iii) Do we have a sufficiently reliable method of detecting *T. vivax* in the blood of cattle? Due to the fact that this trypanosome sometimes causes aparasitaemic phases in animals and might hide in extravascular refugees (Desquesnes & Gardiner 1993), it could exist in greater proportions than those detected in this study. Indeed, a proportion of infections of cattle identified by PCR as *T. vivax* needed 40 reaction cycles to obtain a signal, suggesting also that the primers used lacked sensitivity (Dickin & Gibson 1989) or that *T. vivax* DNA was present in small quantities. It could also be inferred that these primers do not amplify DNA of all *Duttonella* stocks circulating in West Africa (Solano *et al*. 1997), as suggested by a recent work in a wildlife area of Burkina Faso (T. Lefrançois *et al*. unpublished). Perhaps new *T. vivax* primers isolated from the DNA coding for recently described antigen would help to clarify these problems (Masake *et al*. 1997).

To our knowledge, it is the first time that the riverine forest type of *T. congolense* has been identified by PCR in cattle in West Africa. It was only observed in three suspect (negative by microscopic examination) animals, and was associated with *T.c.* savannah in two out of these three cases. Its infection rates were significantly lower in cattle than in tsetse, particularly when compared with *T.c.* savannah-type infection (*T.c.* savannah type was two times more frequent than *T.c.* riverine forest type in tsetse, when it was 20 times more frequent in cattle). This suggests a difference in the transmission of the two taxa. In the present study, the *T.c.* riverine forest type seemed to mature easily in *G. tachinoides*, but not in *G.p. gambiensis*. In a previous work in the Gambia (McNamara & Snow 1991), the riverine forest type of *T. congolense* had been found in *G.p. gambiensis* living in relic forest areas. The forest type seemed to be more frequently transmitted by the riverine species (*palpalis* group), whereas the savannah-type parasite would be confined to the *morsitans* group flies (Reifenberg *et al*. 1997b). In the present work these associations do not appear so clearly. Genetic studies on tsetse at the population level would eventually add some new insights for these host–parasite relationships (Solano *et al*. 1997b). Studies on the compared pathogenicities of trypanosome subgroups could also help to better understand the epidemiology of animal trypanosomiasis in West Africa.

Looking at the differences between the western and the eastern part of the area (Table 3), one can see that epidemiology of trypanosomiasis strongly differs between the two parts that are separated by a few kilometres. The western part comprises high overall infection rates in both tsetse, most of them not being identified by PCR, with the two tsetse species feeding more readily on reptiles thus being less 'dangerous'. In the eastern part, the infected flies are less numerous, but due to the high proportion of bloodmeals on cattle, and of identification of mature infections with known pathogenic trypanosomes, the risk seems as high as in the western part. This stresses the usefulness of a spatial approach to determine the trypanosomiasis risk in a given area. Other information on the human pressure, cattle distribution, and ecological data could help to design a model of the epidemiological situation in this part of West Africa.

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T. Lefrançois, P. Solano, S. de La Rocque, J. M Reifenberg and Z. Bengaly collected the biological material and performed the PCR analyses at the CIRDES (Centre international de Recherche-Développement sur l'Élevage en Zone subhumide) in Burkina Faso (West Africa); I. Kabore performed the bloodmeal analyses. This work represents a part of a multidisciplinary study on the spatialization of the trypanosomian risk directed by D. Cuisance (CIRAD–EMVT France).

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Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso

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Abstract

African animal trypanosomoses constitute the most important vector-borne cattle diseases in sub-Saharan Africa. Generally it is considered that there is a great lack of accurate tools for the diagnosis of the disease. During a trypanosomosis survey in the agro-pastoral zone of Sideradougou, Burkina Faso, 1036 cattle were examined for trypanosomes using microscopy. The PCR was applied on a subset of 260 buffy-coat samples using primers specific for *Trypanosoma congolense* savannah and riverine-forest groups, *T. vivax*, and *T. brucei*. Parasitological examination and the molecular technique were compared, showing a better efficiency of the latter. In the near future, the PCR is likely to become an efficient tool to estimate the prevalence of African trypanosomoses in affected areas.

Keywords: Cattle-protozoa; PCR; *Trypanosoma* spp.; Diagnosis; Burkina Faso

1. Introduction

African animal trypanosomoses are considered as the most important cattle disease transmitted by vectors in sub-Saharan Africa (FAO, 1994). Non polluting control methods are

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currently used and can prove successful under certain conditions (Bauer et al., 1995). However, these methods require a thorough knowledge of the epidemiological situation, which is time and labour consuming. Until now, there has been no reliable diagnosis test able to assess the true prevalence of trypanosomoses in cattle: the classical microscopical examination, either by the Woo technique (Woo, 1970) or by the buffy-coat method (Murray et al., 1977), lacks sensitivity (1000 tryps/ml). Antibody-detection ELISA has the advantage of being economic and is applicable at a large scale, but does not differentiate between past and present infections, nor distinguish between species of trypanosomes. Moreover the test is not suitable for the diagnosis of an individual animal. Antigen-detection ELISAs have been shown to lack specificity and also sensitivity (Desquesnes, 1996). The polymerase chain reaction (PCR), using primers designed from satellite genomic DNA sequences specific of different taxonomic groups, has proved the most sensitive and specific experimental technique to detect trypanosomal DNA in either the vector or the host (Moser et al., 1989; Masiga et al., 1992; Majiwa et al., 1994; Solano et al., 1995; Reifenberg et al., 1997; Lefrançois et al., 1998). However, PCR has been scarcely applied to assess the prevalence of trypanosomosis on field samples, due to its reputed time-consuming and prohibitive cost aspects, and the requirement for technical expertise.

In the agro-pastoral zone of Sideradougou (Southwestern Burkina Faso), a multidisciplinary study has been undertaken, the aim of which was to identify the key factors affecting the presence of tsetse flies. All data on entomology, parasitology, livestock, hydrographic network, environment, land use, have been geographically referenced and included in a Geographic Information System, in order to deduce indicators of areas at higher risk of transmission (de La Rocque et al., 1998). Tsetse had been completely eradicated from the area some years ago by the combined use of impregnated screens and sterile insect technique (Politzar and Cuisance, 1984). After the program had been stopped, tsetse reinvaded the area and are now occupying the galleries all along the hydrographic network in the area (de La Rocque et al., 1998). A total of 70 000 cattle were estimated to occupy the area in 1988 and a new estimation is in preparation (Michel et al., 1999). A rapid and reproducible evaluation of the prevalence of trypanosomosis is required together with other key indicators, in order to decide on appropriate strategic interventions for a sustainable control of the disease.

The objective of the study was to apply the PCR technique on a field survey for the first time in West Africa in order to assess the prevalence of trypanosome infections in cattle, with a minimum possible cost and maximum speed, and to compare the results obtained on a random sample with those using a parasitological method.

2. Material and methods

2.1. Study area

The survey was made in November 1997 in the northern part of the agro-pastoral zone of Sideradougou (see Fig. 1). An exhaustive and georeferenced survey of the geographical distribution of cattle had been made in this northern part which lead to a count of 16 600

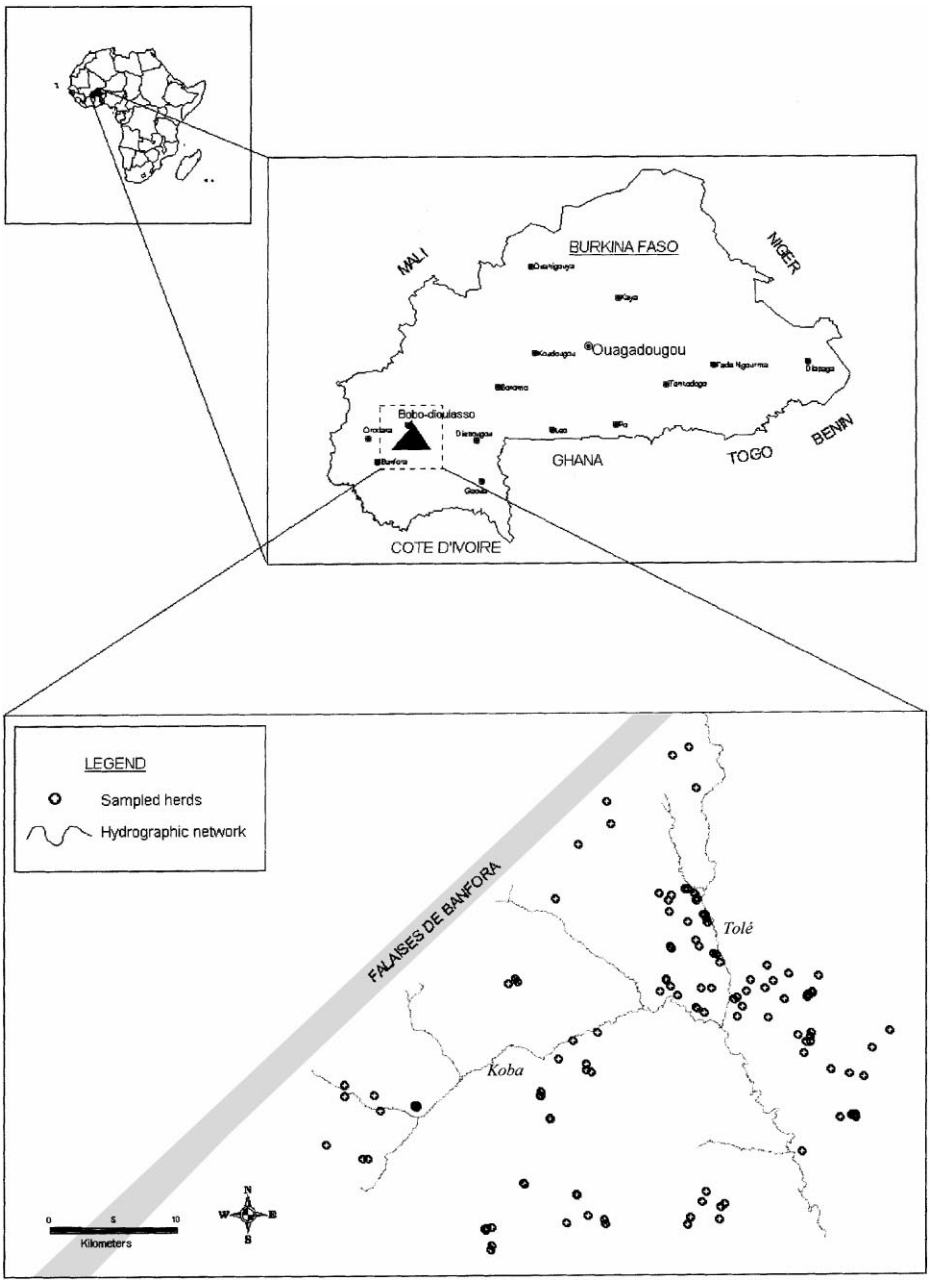

Fig. 1. Map of the Sideradougou area, Burkina Faso, and geographic location of the sampled herds.

Table 1
Polymerase chain reaction primers used in the study

Primers	Reference	Specificity	Amplification Size product
TBR 1, 2	Moser et al., 1989	*T. brucei s. l*	177 bp
VOL 1, 2	Dickin and Gibson, 1989	*T. vivax*	180 bp
TCS 1, 2	Majiwa et al., 1993	*T. congolense* savannah type	320 bp
TCF 1, 2	Masiga et al., 1992	*T. congolense* riverine-forest type	350 bp

heads of cattle (Michel et al., 1999). The Sideradougou area harbours a typical southern Sudanian vegetation with riverine forests along the hydrographic network. The zone is crossed by two majors rivers, the Koba and the Tolé, and a secondary hydrographic network appears during the rainy season (June to October). Riverine tsetse flies of the *palpalis* group (*Glossina tachinoides* and *G. palpalis gambiensis*) constitute the potential vectors of trypanosomes in the area.

2.2. Epidemiological survey and sampling methods

A representative stratified random sample (e.g. Cochran, 1963) of 1036 cattle was selected on the basis of the above mentioned exhaustive inventory (see Fig. 1), to allow an estimate of the prevalence in the total sample with a maximum error of 6%.

From each animal, 5 ml of blood were collected from the jugular vein in a heparinised tube, and brought back to the field camp which was in the immediate vicinity. Two capillary tubes were prepared from each blood sample. The first tube was used to measure haematocrit and to detect trypanosomes by examination of the buffy-coat using dark-ground microscopy (Murray et al., 1977). The second tube was broken just under the limit between the buffy-coat and the red cells, and the buffy-coat was mixed with 30 μl of sterile distilled water in a 0.5 ml eppendorf tube. After agitation, samples were frozen before further analysis.

Out of the total population of cattle examined, a randomly drawn subset of 260 buffy-coat samples was analysed for detection of trypanosomal DNA by PCR.

Trypanosome prevalences were calculated with normal confidence intervals.

2.3. DNA amplifications

The 0.5 ml eppendorf tubes containing the buffy-coat samples in sterile distilled water were brought back to CIRDES where all subsequent technical procedures were done. First, 100 μl of 1% aqueous solution of Chelex® (BIORAD) were mixed with the buffy-coat of each sample and the solution was incubated for 1 h at 56°C and 30 min at 95°C (Walsh et al., 1991). Thereafter each tube was centrifuged for 2 min at 15 000 g and stored at −20°C.

Primer sets specific for *Trypanozoon*, the savannah and riverine forest types of *T. congolense*, and *T. vivax* were used (see Table 1). All these primer sets have been evaluated for sensitivity and specificity by the authors mentioned as reference, and also by others (see for example Solano et al., 1995). Standard PCR amplifications and electrophoresis were carried out as previously described (Solano et al., 1995) except that standard PCR mixes were done simultaneously for 50 samples and included two negative controls: the first was

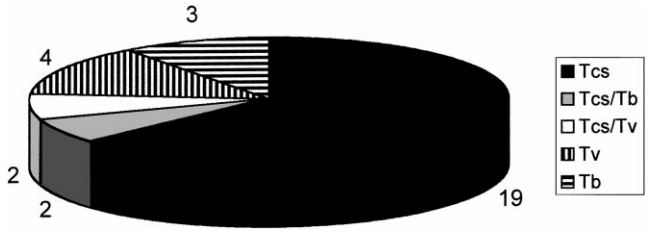

Fig. 2. Taxonomic identifications of trypanosome infections detected by PCR in cattle. Note: Tcs is *Trypanosoma congolense* 'Savannah' type; Tv is *T. vivax*; Tb is *T. brucei*.

placed in 25th position, and the second at the end. A positive control with reference DNA of each trypanosome type was also included in each PCR assay. Amplification products were resolved in 1.6% agarose gels (14 cm), stained with ethidium bromide. When the amplification reaction gave a signal of the expected size according to the set of primers used without any signal in the negative controls, the infection was considered to be characterised.

3. Results

3.1. Parasitological results

The parasitological prevalence was 5.3(±1.3)% in the total sample of 1036 individuals (55 positive cases). On the 260 selected individuals, it was 4.2(±2.4)% (11 positive cases). These two numbers were not statistically different.

Out of these 260 animals, most of the infections found by microscopical examination were attributed to *T. congolense* (7 cases), followed by *T. vivax* (3 cases) and *T. brucei* (1 case).

3.2. PCR results

The overall prevalence obtained on the subset reached 11.5(±3.9)%. Out of the 30 animals found infected by PCR, *T. congolense* riverine-forest type was never observed. *T. congolense* savannah type was more frequently detected than *T. vivax* and *T. brucei* (see Fig. 2), as it was found in approximately 80% of the infected animals. Four individuals had an infection with two different trypanosomes (2 *T. congolense* savannah/*T. vivax* and 2 *T. congolense* savannah/*T. brucei*).

3.3. Comparison between parasitological and PCR results

The χ^2 test on the original data set showed a significant difference between the prevalence using PCR and the parasitological one ($p < 0.01$). Indeed it represented more than two times the prevalence detected parasitologically (11.5% vs. 4.2%). The main difference consisted in the number of positive cases of *T. congolense* savannah and *T. brucei* (see Fig. 3), which dramatically increased using the molecular technique.

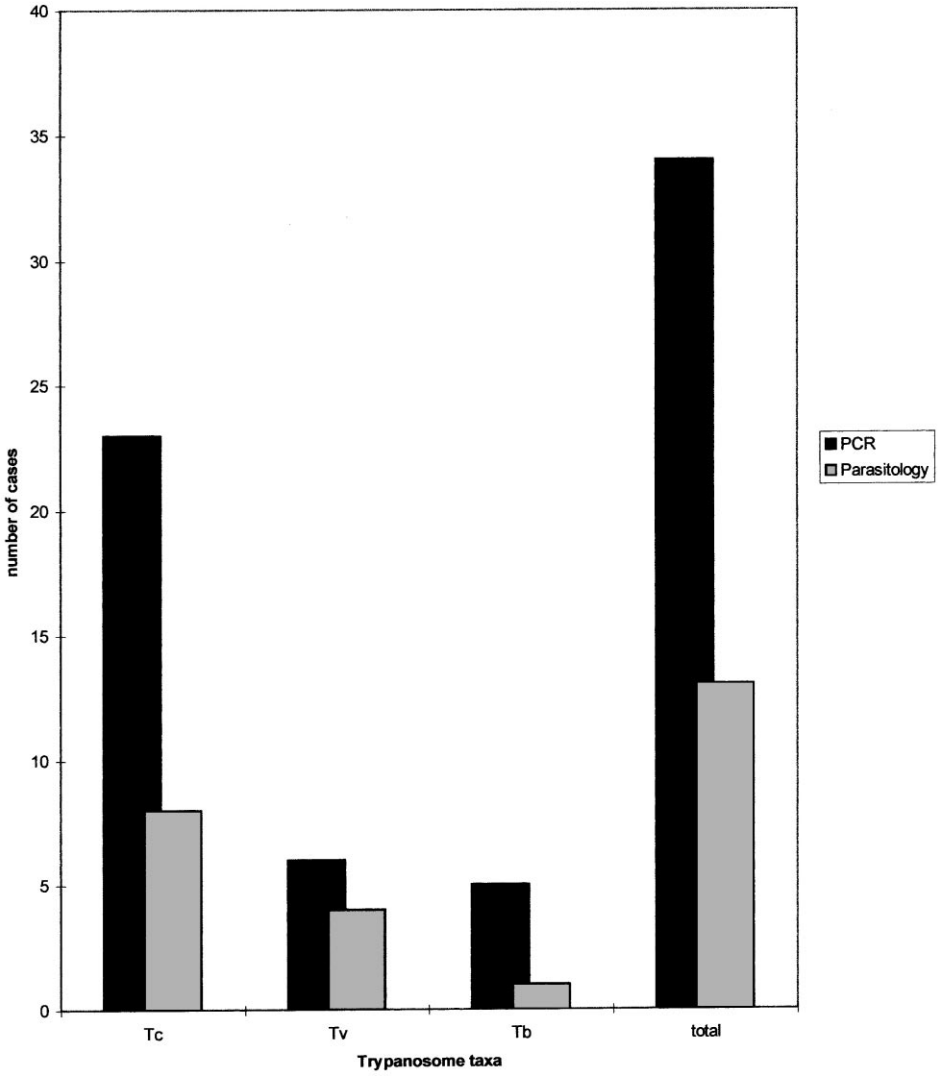

Fig. 3. Number of positive infections detected by PCR and by microscopical examination. Note: the number of infections is higher than the number of cases because of the four cases of mixed infections detected by PCR.

Out of the 11 animals which had been found infected by microscopical examination, PCR gave interpretable signals on 10, which represents a 91% identification.

On the other hand, the PCR gave clear amplification signals on 20 animals not detected positive by parasitological examination. This highlights the difference between the two techniques because of the 30 cases positive by PCR, two-thirds (most of which had a haematocrit value below 25%) were negative using the parasitological technique. Furthermore, PCR allowed the accurate diagnosis of four mixed infections, which could not be detected by parasitological examination.

At no time during the study, the negative controls showed a visible signal. Moreover, all the primer sets used had been carefully checked for absence of cross-amplifications (see also Majiwa et al., 1994; Solano et al., 1995).

4. Discussion

The PCR technique using primers designed on repetitive genomic DNA sequences of trypanosomes had already been used for epidemiological purposes. However, such investigations focused essentially on the identification of trypanosomes in tsetse flies (McNamara et al., 1995; Masiga et al., 1996; Solano et al., 1996; Reifenberg et al., 1997), and to a much lesser extent in cattle. The technique has been verified on blood samples of experimentally infected animals, confirming its higher sensitivity and specificity when compared to parasitological techniques (Moser et al., 1989; Clausen et al., 1998). In the present work, we used the PCR technology as a diagnosis tool to assess the prevalence of animal trypanosomosis on samples collected in the field, in West Africa, at the geographic scale of an agro-pastoral zone.

Using the PCR, a significantly higher prevalence was obtained than by classical parasitological examination. This was due to the higher sensitivity of the molecular technique which is able to detect less than one trypanosome per ml under experimental conditions (Moser et al., 1989). Such prevalence differences as obtained by the two techniques would have a significant impact on the strategy selected for the control of the disease in affected areas. Interestingly, we demonstrated that analysing a random sample of cattle, the PCR technique could be applied in a rapid and simple way (three weeks of work for one technician to analyse the 1040 PCR samples: 260 individuals * 4 primer sets). We showed, as others did previously (Penchenier et al., 1996; Katakura et al., 1997), that no labour and time-consuming DNA extractions using classical protocols were anymore needed for epidemiological surveys. By using chelating resins, the available DNA in the samples could be properly amplified. From a technical point of view, we believe that PCR applied on buffy-coat samples seems more promising than on whole blood extracted from filter paper, as the concentration of trypanosomes is higher in the buffy-coat (Woo, 1970; Murray et al., 1977). Moreover the technique here appeared quite economic (0.5 US$ per head of cattle) when compared to previous studies (for review, see Desquesnes and Tresse, 1996). Progress in lowering the costs of reagents used and minimising time spent on the PCR (Mai et al., 1998), will facilitate the diagnosis of field samples.

One animal presented an infection (diagnosed by microscopy as *T. congolense*) that was not recognised by the sets of primers used. This could reflect either a technical problem, or the presence of trypanosomes which could not be recognised by the primers used, as has been reported previously (Katakura et al., 1997). Nevertheless, the technique used here appeared satisfactory. It should also be noticed that in the present survey, 12.4% of the 1036 cattle had a haematocrit value below 25% (such a value is a sign of anaemia): this percentage is consistent with the trypanosome prevalence found by PCR. Among the individuals showing such a low haematocrit value, upto 40% were found infected with trypanosomes using PCR (data not shown). These results confirm findings in this study area reported elsewhere (Lefrançois et al., 1998) and underline

that the haematocrit value is a good indicator of trypanosomosis in cattle (Féron et al., 1987).

5. Conclusion

Taking into account current views on sustainable control of African trypanosomoses (Hendricx et al., 1997), a need arises for tools that can assist in a rapid appraisal of the parasitological situation in agricultural and livestock areas: the use of the PCR to assess trypanosome prevalence in cattle seems a valuable tool for this purpose.

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Microsatellite DNA markers reveal genetic differentiation among populations of *Glossina palpalis gambiensis* collected in the agro-pastoral zone of Sideradougou, Burkina Faso

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Abstract

Intraspecific genetic variability of *Glossina palpalis gambiensis* in the area of Sideradougou, Burkina Faso, was studied using polymorphic microsatellite DNA markers. This genetic study was combined with other epidemiological information on the same tsetse: blood-meal identification, dissection of tsetse and molecular characterization of the trypanosomes detected. There was significant genetic differentiation among flies caught only a few kilometers apart, within the same riverine habitat. These distinct subpopulations were also differentially infected by trypanosomes. In part of the study area, a Factorial Correspondence Analysis undertaken on the genotypes allowed us to detect a Wahlund effect, suggesting the presence of tsetse originating from different source populations coming from two distinct drainage systems. The apparent structuring of populations of *G. palpalis gambiensis* is discussed relative to appropriate strategies to control African Trypanosomosis.

Keywords: Burkina Faso, epidemiology, *Glossina palpalis gambiensis*, microsatellite, population genetics, trypanosomosis.

Introduction

Genetic variation among vector populations probably affects the transmission of many parasitic diseases at a macro-geographic level (Lanzaro & Warburg, 1995), but little information is available to assess the spatial scale of this phenomenon. Recent developments in techniques for assessing variation at highly polymorphic microsatellite DNA markers (Tautz & Renz, 1984; Estoup *et al*., 1993; Weber & Wong, 1993) have provided us with new tools to investigate the question. *Glossina palpalis gambiensis* VanderPlank 1949, is a riverine West African tsetse fly that transmits trypanosomes causing both human and animal African Trypanosomoses. Improving our understanding of the genetics of the vectorial capacity of tsetse is clearly a worthy endeavour (Janssen & Wijers, 1974; Maudlin, 1980; Reifenberg *et al*., 1997; Kazadi *et al*., 1998). Similarly, genetic studies may provide useful information on the potential for large-scale tsetse control particularly in terms of Sterile Insect Technique (Cheng & Aksoy, 1999; Dale *et al*., 1995; Vreysen *et al*., 1998).

Tsetse were eradicated from the agro-pastoral zone of Sideradougou, south-west Burkina Faso, 15 years ago through the combined use of insecticide-impregnated screens and Sterile Insect Technique (Politzar & Cuisance, 1984). In recent years, tsetse have reinvaded the area, and now once again inhabit the gallery along major rivers. As part of a larger multidisciplinary work to understand the key factors determining the presence of tsetse flies (de La Rocque & Cuisance, 1997), we undertook basic studies on the relationships between microgeographic genetic variation in a tsetse species and other risk factors related to disease transmission.

We had previously isolated three microsatellite polymorphic loci from a genomic bank of captive-bred *G. palpalis gambiensis* (Solano *et al*., 1997). Here we report the use of these loci to characterize tsetse captured in the area of Sideradougou, in relation to the microgeographic epidemiology of trypanosomosis.

Results

Entomology

A total of 620 *G. palpalis gambiensis* was caught with apparent densities of eight to thirty-one flies/trap/day in the west, and up to five in the east. The mean sex ratio of the tsetse was 1.4 males per female in the west, and it was 0.6 in the east (Fisher's exact test: $P = 0$).

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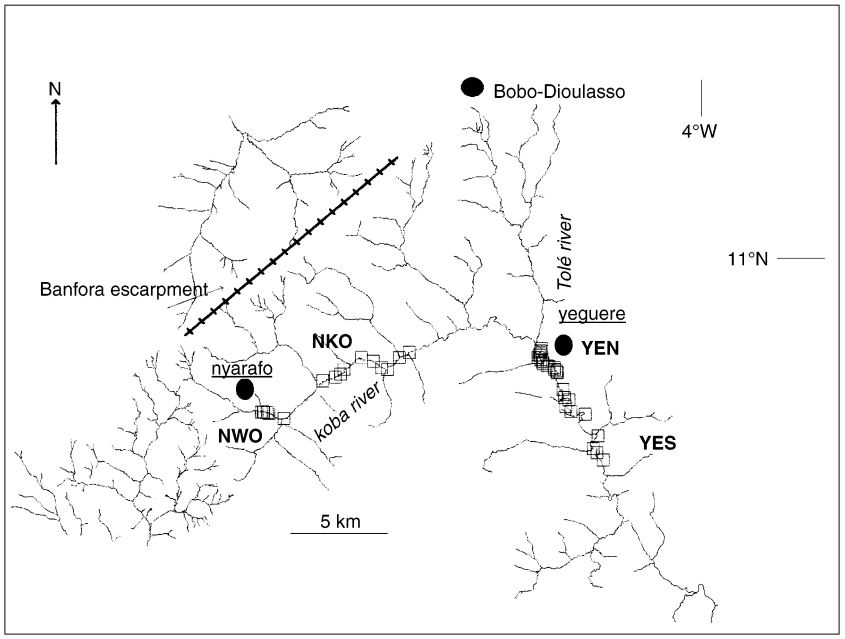

Figure 1. Map of the study area and geographical location of the traps (□) and subsamples (NWO, NKO, YEN, YES).

Epidemiological results

From 215 dissected flies, mean parasitological infection rates differed significantly between the areas: 20.3% (west) and 3.5% (east) (Fisher's exact test: $P = 0.0024$).

Using PCR to characterize the trypanosomes in the flies, some infections in the west could be characterized as *T. vivax* (40% of infected flies) or *T. congolense* «forest type» (6%). Surprisingly large numbers of infections (54%) did not react with any primers. These infections were localized only in the midgut of the flies. Because reptiles constituted the main food source (eight of twenty-one identified bloodmeals), this lead us to hypothesize that reptilian trypanosomes could account for these many infections. To date, specific primers for reptilian trypanosomes are not generally available (but see Gouteux & Gibson, 1996). Tsetse in both areas also fed on Suidae, followed by other species including cattle and man.

Infection rates were very low in the East, only two infected *G. palpalis gambiensis* were found; these harboured *T. vivax*.

Genetic analyses at the scale of the agropastoral zone

Among 201 analysed tsetse females, a total of twelve and seventeen alleles was recorded, respectively, at loci *Gpg55.3* and *Gpg19.62*. Seven subsamples (i.e. populations) were defined according to their geographical location and year of capture (Fig. 1).

No significant linkage disequilibrium was detected between loci in any of the subsamples.

The overall *Fis* value (0.163) across these two first microsatellite loci indicated a strong departure from panmixia,

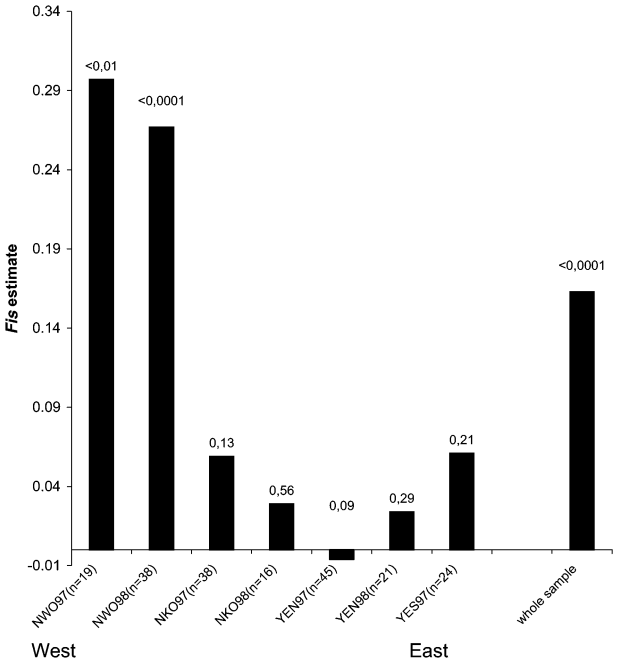

Figure 2. Graph showing within-sample heterozygote deficiency and its significance in each subsample. The subsamples are presented sequentially from the west to the east of the area and are plotted against the *Fis* estimate. Above each sample is represented the probability associated with the test of significance of the *Fis*. The number of females of each subsample is indicated in parentheses.

but this observation was dependent on location. Positive values of *Fis* (within sample heterozygote deficiency) were common only in the west. In the east, we could not reject the null hypothesis of panmixia (Fig. 2).

Table 1. Matrix of pair-wise *Fst* measured between the seven studied samples

	NWO97	NKO97	NKO98	NW098	YEN97	YES97	YEN98
NWO97	/	0.0211	−0.0086	−0.0085	**0.0590**	**0.0597**	**0.0831**
NKO97		/	0.0158	**0.0610**	**0.0810**	**0.0713**	**0.1399**
NKO98			/	0.0119	0.0213	0.0236	0.0627
NWO98				/	**0.0995**	**0.1077**	**0.1163**
YEN97					/	−0.0093	0.0354
YES97						/	0.0355
YEN98							/

Significant values after Bonferonni procedure are in bold (Bonferonni level is $\alpha' = \alpha/21$; $\alpha = 0.05$; so $\alpha' = 0.0024$).

The subsamples are presented from the west (four subsamples) to the east (three subsamples). Their names show the location and the year of capture. NOW = Nyarafo Wood; NKO = near Nyarafo on the Koba river; YEN = north of Yeguere; YES = south of Yeguere.

The overall *Fst* value was highly significant (*Fst* = 0.059, *P* < 0.0001) when measured at the scale of the entire area. This indicated genetic differentiation between subsamples (Table 1). The highest values of *Fst* were observed between western and eastern subsamples. Comparisons within each year were also significant (1997: *Fst* = 0.051, *P* < 0.0001; 1998: *Fst* = 0.072, *P* < 0.0001). When samples were pooled according to origin (i.e. west and east), the *Fst* estimate was 0.07 (*P* < 0.0001). This value suggests an exchange rate equivalent to three to four reproducing flies per generation (according to the formula: Nm = (1 −*Fst*)/4**Fst*, of the measure of gene flow in an island model at mutation/drift equilibrium; Wright, 1969).

Genetics on the flies collected in Nyarafo, in the west

A strong *Fis* value was found in the samples collected in the gallery forest close to Nyarafo village in the western part. The third locus (*Gpg69.22*) was then used to amplify DNA from the tsetse collected in this Western part, i.e. NWO97, NKO97 and NWO98, which represent a total of ninety-seven females. Additional data at this locus confirmed the previously found strong *Fis* value in this Nyarafo area (across the three loci *Fis* = 0.20, *P* < 0.0001).

A Factorial Correspondence Analysis was then undertaken on the multilocus genotypes of the three loci to detect a possible Wahlund effect (population subdivision). After removal of flies carrying rare alleles, several groups were readily apparent in the plot of results from the FCA. Group C defined by axis 2 contained only six flies. It can be seen in Fig. 3 that axis 1 (which represented 16% of the total variance out of 14 axes) helped to more or less arbitrarily define two groups, A and B. These two groups may be seen as belonging to a single group, but the following suggest that a big proportion of group A's individuals and a big proportion of group B's individuals do not belong to the same population. Indeed, the mean *Fis* values for each locus decreased when calculated separately for the two groups A and B (Table 2). The mean value (*Fis* = 0.034) in the two separated groups A and B was no longer significant. This means that subsamples A and B did not represent a random subsampling of the original sample. These results (i.e. the FCA and the decrease of the *Fis* in each of the two groups at all loci) were consistent with the hypothesis that the initial *Fis* did not differ from 0 and that there was a Wahlund effect in the sampled flies. No significant linkage disequilibrium could be detected in the sample.

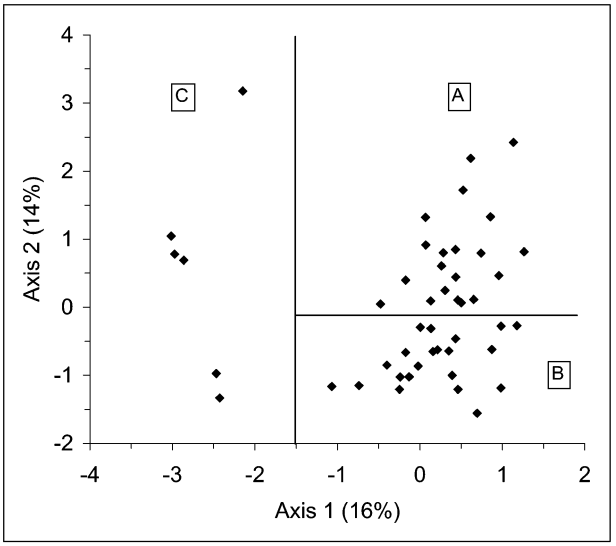

Figure 3. Results of the Factorial Correspondence Analysis undertaken on the flies collected in the western part (Nyarafo).

Table 2. Decrease of the *Fis* value between the total sample and the two groups (A and B) deduced from the Factorial Correspondence Analysis carried out on the flies from Nyarafo

Fis value	Total sample (A + B)	Sample A-Sample B
Locus *Gpg55.3*	0.09	−0.07
Locus *Gpg19.62*	0.12	0.03
Locus *Gpg69.22*	0.12	0.07
Across loci	0.20	0.034

Taking into account these two groups of individuals (A and B) occurring in sympatry in the western side of the area in the sacred wood of Nyarafo, the population differentiation remained significant compared to the eastern zone.

Discussion

In this work, the study of *G. palpalis gambiensis* population structure was undertaken simultaneously with other epidemiological factors, i.e. bloodmeals preferences and molecular identification of the trypanosomes found in these tsetse. Particular attention was made to simplify protocols for DNA extraction, in order to enable such work to be carried out in the field. The combined study of different components of the pathogenic system should allow a better understanding of the trypanosomosis risk in a given area (de La Rocque *et al*., 1998).

We have found significant cryptic substructuring of local tsetse populations over just a few kilometres in a gallery forest habitat along a given riverine system. The low rates of gene flow suspected between the western and eastern tsetse populations of the Koba river might be explained by the relatively harsh habitat conditions separating these two parts: water is not present during the whole year and the vegetation is sparse. Indeed only a few tsetse were caught in this place during the extensive survey of 1996, and among these hardly any teneral (i.e. young) flies (de La Rocque & Cuisance, 1997). Still comparing western and eastern situations, differences were also found in the sex ratio of the captured tsetse, their infection rates and the identity of the trypanosomes that infected them. These differences confirmed those already reported in another study in the same area on more than 2000 tsetse: 'western' flies infected by trypanosomes were found much more often, and the hypothesis was that these infections could be attributed mainly to reptilian, non pathogenic trypanosomes (de La Rocque *et al*., 1998; Lefrançois *et al*., 1998). Conversely, the 'eastern' flies, despite being less infected, fed much more on cattle, and were infected mostly by *T. vivax*, a trypanosome of major veterinary importance (de La Rocque *et al*., 1998; Lefrançois *et al*., 1998).

Hence, along the drainage system of the Koba river, genetic structuring of the populations of *G. palpalis gambiensis* was consistent with an 'epidemiological structuring'. Genetically different populations of the vector were associated with different epidemiological patterns in the area. Obviously, various other factors could also play a role in the efficiency of the transmission of the disease, such as cattle distribution and density (Michel *et al*., 1999), land use, water resources and vegetation (Cuisance & de La Rocque, 1998). Other genetic studies on tsetse have also demonstrated genetic differences among populations, e.g. through isoenzyme analyses of *Glossina pallidipes* in eastern and southern Africa (Nesbitt *et al*., 1990; Kence *et al*., 1995; Krafsur *et al*., 1997), or through the use of the same microsatellite loci between geographically distant populations of *G. palpalis gambiensis* (Solano *et al*., 1999), however these studies have worked on a macrogeographic scale, or have compared populations from ecologically distinct areas.

In the present study, as a second level of structuring, a highly significant heterozygote deficiency was observed, involving the three microsatelllite loci on the flies collected in the Western part, near Nyarafo. It should be noted that, to explain a strong *Fis*, technical and/or biological factors might be involved.

From a technical point of view, the existence of null alleles at microsatellite loci has already been reported, i.e. non-amplification of alleles at microsatellite loci due to mutations in the flanking sequences (Callen *et al*., 1993; Paetkau & Strobeck, 1995; Dumas *et al*., 1998). In the present study, both microsatellite loci *Gpg55.3* and *Gpg19.62* were interpreted to be located on the X chromosome. Thus, if null alleles were the cause of the heterozygote deficiencies, we would have expected the occurrence of null males. However, all the males showed one band. Even in females, the frequency of a null allele accounting for the observed deficiency would be so high that null homozygotes (i.e. females showing no PCR product) would have been expected (Brookfield, 1996), which was not the case. Therefore it is not likely that null alleles could explain the observed heterozygote deficiencies.

Biological reasons could also account for the observed high *Fis* values, such as the presence of a Wahlund effect (sampling two independent gene pools). Indeed the FCA, which was used here as a tool to visualize possible structuring, suggested the occurrence, in sympatry, of two (or more) genetically distinct groups of tsetse in the western part of the area in the sacred wood close to the village of Nyarafo. It is not stated here that all the individuals of group A and all individuals of group B belong to different populations. What appears from the analyses is that the strong original *Fis* found in the total sample comes from a Wahlund effect, because if these two groups did not reflect any biological reality, there is no reason why the *Fis* woud have changed. Here, this Wahlund effect can be the consequence of two phenomena. First, *G. palpalis gambiensis* could represent a complex of (at least) two species with restricted or null gene flow. Second, the area may have been colonized by flies coming from different source populations following the end of tsetse control operations (Politzar & Cuisance, 1984). Our study area is just under Banfora escarpment (see Fig. 1), above which flows the basin of the Comoe river. Two different drainage systems join here, i.e. the Comoe and the Koba rivers (the latter belonging to the Mouhoun basin), which might represent the providers of these different tsetse populations.

Table 3. Characteristics of the three microsatellite loci isolated from *Glossina palpalis gambiensis*

Microsatellite locus	Core microsatellite sequence	Annealing temperature	Number of alleles in the whole sample	Frequency of most common allele
Gpg55.3	$(GT)_{14}(GC)(GT)_4$	50 °C	12	0.79
Gpg19.62	$(GT)_{12}$	50 °C	18	0.49
Gpg69.22	$(TA)_{10}$	48 °C	8*	0.60

*Locus *Gpg69.22* was scored on ninety-seven females from the western area.

Because this tsetse species moves preferentially along riverine networks and needs high humidity to survive (Buxton, 1955), the geography of the area provides the most likely explanation for population substructuring.

It would be of interest to know if the genetic differences at the intraspecific level pointed out in the present work could lead to different vectorial competences (Maudlin, 1980; Reifenberg *et al.*, 1997; Moloo *et al.*, 1998).

The lack of evidence for any linkage disequilibrium is not definitive in our study due to the low number of loci analysed. The third group of the FCA (group C) might also represent individuals having migrated from other localities. However in the absence of clearer information that would come from the analysis of more loci, we cannot draw more precise conclusions. It should be noted that to the author's knowledge, these three microsatellite loci are the only ones that have been described in tsetse flies so far. Nevertheless the information expected from such approaches will contribute in designing future control strategies: genetic studies on vector populations combined with the study of other epidemiological factors will be of help in identifying 'epidemiologically dangerous points' to assess areas at risk for trypanosomosis (Cuisance *et al.*, 1980; de La Rocque, 1997; Hendrickx *et al.*, 2000; B. Bauer & B. Snow, personal communication), and will also help to assess the feasibility of the Sterile Insect Technique in tsetse eradication schemes (Vreysen *et al.*, 1998).

Experimental procedures

Study area

Sideradougou lies in the southern half of Burkina Faso in the Sudan savannah vegetation zone (4°20′W, 10°55′N). Two riverine tsetse species are present in the area: *Glossina palpalis gambiensis* and *G. tachinoides* Westwood 1850. The main drainage system comprises the Koba and the Tolé rivers (Fig. 1). An exhaustive entomological survey was completed in 1996 along 120 km of the main Koba river (de La Rocque, 1997). These data were used to select two areas for detailed investigation in the dry seasons of 1997 and 1998. In each area we deployed biconical (Challier & Laveissière, 1973; Challier *et al.*, 1977) and monoconical (Laveissière & Grébaut, 1990) traps for 2 days, near the village of Nyarafo in the west, and near Yeguere in the east (Fig. 1). These two areas differ in infection rates and in the identity of the trypanosomes in the flies (de La Rocque *et al.*, 1998; Lefrançois *et al.*, 1998).

Field techniques

Each trap site was georeferenced using a GPS and tsetse species, sex and apparent density were recorded.

In the field camp, after removing of the tsetse from the trap, individual *G. palpalis gambiensis* were processed as follows:

- Three legs were removed and stored in dry eppendorf tubes for subsequent genetic analyses.
- The mouthparts, salivary glands and midgut were dissected to detect trypanosome infections by microscope examination (Lloyd & Johnson, 1924).
- The midgut content was collected for subsequent bloodmeal identification (Kaboré *et al.*, 1994).
- When a tsetse was found infected with trypanosomes, each of the three organs was collected separately in eppendorf tubes (dry for the mouthparts, and with 50 µl sterile distilled water for the midgut and salivary glands). This material was used for PCR identification of trypanosomes using DNA primers designed from genomic satellite sequences specific of the different taxonomic groups (Majiwa *et al.*, 1994; Solano *et al.*, 1995).

The techniques for identification of the trypanosomes by PCR and for the bloodmeal origin of the tsetse are described fully in Lefrançois *et al.*, 1998.

PCR reactions at microsatellite loci

At the CIRDES (Centre International de Recherche-Développement sur l'Elevage en zone Subhumide) laboratory in Bobo-Dioulasso, 300 µl of 5% Chelex® (BIORAD, Hercules, CA, USA) chelating resin were added to each tube containing the legs of the tsetse (Walsh *et al.*, 1991; Dumas *et al.*, 1998). After incubation at 56 °C for 1 h, DNA was denatured at 95 °C for 30 min. The tubes were then centrifuged at 12 000 ***g*** for 2 min and were frozen before further handling.

The PCR reactions were carried out in a thermocycler (MJ Research, Watertown, MA, USA) in 50 µl final volume, using 10 µl of the supernatant from the extraction step. Specific primers were designed in the regions flanking the microsatellite core sequences of loci *Gpg55.3* and *Gpg19.62* (as described in Solano *et al.*, 1997). Precise allele sizing on a limited number of samples had already been determined by performing PCR in the presence of (α^{35}-S) dATP, followed by denaturing electrophoresis in 6% acrylamide gel containing 8M urea (Solano, 1998). In the present work, the loci were amplified using the same conditions (annealing temperature was 50 °C) (see Table 3). After PCR amplification, allele bands were routinely resolved on 10% nondenaturing polyacrylamide gels stained with ethidium bromide. Due to logistical constraints, the third locus (*Gpg69.22*) was scored only in Nyarafo (in the western part) in 1997 and 1998. Primers sequences at the three microsatellite loci are described in Solano *et al.*, 1997.

The microsatellite loci *Gpg55.3* and *Gpg19.62* are presumed to be on the X-chromosome, given an absence of heterozygous

males in captive-bred flies (Solano, 1998). We therefore analysed only field-collected females. Captive breeding also demonstrated mendelian inheritance of the alleles at these microsatellite loci and an absence of null alleles (e.g. Callen *et al*., 1993; Dumas *et al*., 1998).

Data analysis on microsatellite loci

In each population, Wright's *Fis* (within sample heterozygote deficiency) and *Fst* (measure of population differentiation) were estimated using Weir & Cockerham's (1984) unbiased estimators (f for *Fis*, θ for *Fst*). These estimators were computed with FSTAT v.1.2 software (Goudet, 1995). For random mating (within samples) or random distribution of individuals (between samples), *F*-values were expected to be zero.

For each locus in each sample, and for all loci, the significance of *Fis* (panmixia) was tested using GENEPOP v.3.1 (Raymond & Rousset, 1995). The probability test described by Guo & Thompson (1992) was used, employing a complete enumeration method (Louis & Dempster, 1987) for loci with up to four alleles, and a Markov chain method (Guo & Thompson, 1992) for loci with more than four alleles. Measuring the deficit in heterozygotes simultaneously in several samples, the multisample extension of the score test described by Rousset & Raymond (1995) was then applied. For this test, the alternative hypothesis was 'H1 = heterozygote deficiency'. The measure of *Fis* and its significance were conducted only on the females, because males were haploid at two loci (i.e. they have only one X-chromosome).

The significance of *Fst* (population differentiation) was assessed using 10 000 permutations of female genotypes among samples (FSTAT; Goudet, 1995). The alternative hypothesis here is 'Fst > 0'.

Linkage disequilibrium was tested by the exact test of GENEPOP for genotypic linkage disequilibrium (Raymond & Rousset, 1995).

To evaluate significance when multiple tests were performed, the sequential Bonferroni procedure was applied (see Rice, 1989).

A Factorial Correspondence Analysis (FCA) was undertaken to explore genetic structure within samples. The analysis was carried out on the multilocus genotypes obtained at the three loci: each individual was characterized for each existing allele by the values 2, 1 or 0 whether it had 2 (homozygotes), 1 (heterozygote) or 0 copies of the considered allele. Individuals were then analysed as active variables using FCA (e.g. Ba *et al*., 1993). The analysis was carried out after eliminating individuals carrying rare alleles (< 5%), because rare alleles tend to hide the existing patterns.

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TRYING TO PREDICT AND EXPLAIN THE PRESENCE OF AFRICAN TRYPANOSOMES IN TSETSE FLIES

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ABSTRACT: Trypanosome infections identified by polymerase chain reaction on field-caught tsetse flies from various locations were analyzed with respect to factors intrinsic and extrinsic to the trypanosome–tsetse association. These factors were then simultaneously analyzed using artificial neural networks (ANNs) and the important factors were identified to predict and explain the presence of trypanosomes in tsetse. Among 4 trypanosome subgroups (*Trypanosoma brucei* s.l., *T. congolense* of the 'savannah' and of the 'riverine-forest' types, and *T. simiae*), the presence of the 2 types of *T. congolense* was predictable in more than 80% of cases, suggesting that the model incorporated some of the key variables. These 2 types of *T. congolense* were significantly associated in tsetse. Among all the examined factors, it was the presence of *T. congolense* savannah type that best explained the presence of *T. congolense* riverine forest type. One possible biological mechanism would be 'hitchhiking,' as previously suspected for other parasites. The model could be improved by adding other important variables to the trypanosome tsetse associations.

Trypanosomes are widespread in Africa, where they cause diseases of medical and veterinary importance, e.g., human 'sleeping sickness' and 'nagana.' Although mechanical transmission by biting flies may take place, these protozoan parasites are mainly transmitted cyclically by tsetse flies (*Glossina*). In epidemiological studies on trypanosomosis, it is important to know the infection rates of tsetse flies and the kinds of trypanosomes they carry. These parameters are part of the vectorial capacity of tsetse, which is a main component of disease transmission risk and which depends on several factors (Molyneux, 1980; Welburn and Maudlin, 1999). These factors can be grouped into intrinsic risk factors (restricted to trypanosome–*Glossina* interactions, so called vectorial competence) and extrinsic risk factors (ecological, most of which remain to be identified) (Reisen, 1988; Reifenberg, Cuisance et al., 1997). Routine identification of trypanosomes in tsetse has largely relied on dissection and microscopic examination of tsetse organs (Lloyd and Johnson, 1924), but this method is only accurate to subgenus. Moreover, mixed infections in a single fly (for example of *Nannomonas* Hoare, 1964, and *Duttonella* Chalmers, 1918) cannot be detected by this method; neither can immature midgut infections be distinguished; nor can infection with a few parasites be detected by microscopy. Polymerase chain reaction (PCR), using repetitive DNA sequences specific for each species or subgroup of trypanosome (Moser et al., 1989; Masiga et al., 1992; Majiwa et al., 1994), overcomes problems of sensitivity and specificity associated with the traditional methods of identification. PCR has been used extensively for accurate identification of trypanosomes in naturally infected tsetse in several African countries (McNamara et al., 1995; Solano et al., 1995, 1996; Masiga et al., 1996; Woolhouse et al., 1996; Reifenberg, Solano et al., 1997; De La Rocque et al., 1998; Lefrançois et al., 1998, 1999; Morlais et al., 1998).

In the present study, the results of several studies were compiled in which trypanosomes were identified in the midgut of wild tsetse using PCR. The objective was to determine the most important factors explaining the occurrence of trypanosomes in tsetse flies, which may aid in predicting disease occurrence, reemergence, or resurgence.

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MATERIAL AND METHODS

Composition of data set

Our investigations focused on midgut infections only, because the establishment of a trypanosome infection in tsetse and its maturation may depend on distinct factors (e.g., Maudlin et al., 1991). Only the presence of the trypanosomes was taken into account, not their absence (since trypanosomes not recognized by the sets of primers used may exist).

A total of 256 PCR-identified infections was analyzed. This data set represents a total of 4,885 field-dissected flies (prevalence varied between 0 and 20%). The data were taken from 7 published sources that report PCR identification of trypanosomes found in the midgut of 5 tsetse species or subspecies of major medical or veterinary importance in West Africa (McNamara et al., 1995; Solano et al., 1995, 1996; Masiga et al., 1996; Reifenberg, Solano et al., 1997; Lefrancois et al., 1998, 1999). The 4 trypanosome taxonomic subgroups considered reflect the most widely used PCR primer sets in tsetse fly surveys and were all used in the above-cited references. They are *Trypanosoma congolense* (Broden, 1904) savannah type, *T. congolense* riverine-forest type, *T. simiae* (Bruce, 1912), and *T. brucei* s.l. Dutton, 1902. *Trypanosoma vivax* Zieman, 1905, could not be included because the development of this parasite is restricted to the mouthparts of the tsetse fly. Other studies that used this methodology in other parts of Africa could not be incorporated in this present investigation, owing to different sampling techniques which could have biased the analyses; for example, Woolhouse et al. (1996) did not look for *T. brucei* in the dissected flies and Morlais et al. (1998) did not look for *T. congolense* savannah type.

Explanatory variables

Variables studied, both intrinsic and extrinsic to the vector parasite association, are listed in Table I. As intrinsic variables, the tsetse taxa included represent those of the *palpalis* and *morsitans* groups that are important vectors of both human and nonhuman trypanosomoses. For phylogenetic position of tsetse, 3 categories were defined that represent discrete levels of genetic distances between taxa. Category A separates tsetse of *Glossina* Zumpt, 1935 (*morsitans* group, *Gms* and *Gl*), from those of *Nemorhina* Robineau-Desvoidy, 1830 (*palpalis* group, *Gpp*, *Gpg*, and *Gt*) (see Table I for abbreviations). Then, category B within the *palpalis* group separates *Gt* from both *Gpp* and *Gpg*; category C, at the within-species level, separates *Gpp* from *Gpg*. Because various trypanosome taxa may occur together in a given tsetse midgut (e.g., Solano et al., 1995; Woolhouse et al., 1996), the presence of trypanosomes other than the one under analysis was entered as a variable.

As extrinsic variables, the tsetse habitat characteristics were those reported in the literature (see, e.g., Buxton, 1955; Laveissière, 1986). The tsetse distribution range was estimated as the number of countries where a given tsetse taxon occurs (Brunhes et al., 1999). For bloodmeal

TABLE I. Explanatory variables included in the study. A random variable was also included (see text).

Variable	Composition	Type*
Tsetse taxa	*Glossina palpalis palpalis* (*Gpp*) *G. palpalis gambiensis* (*Gpg*) *G. tachinoides* (*Gt*) *G. longipalpis* (*Gl*) *G. morsitans submorsitans* (*Gms*)	Intrinsic
Phylogenetic position of tsetse taxa	Categories A, B and C	Intrinsic
Trypanosome taxa	The 3 other taxa than the one under analysis	Intrinsic
Tsetse habitat	Savannah, humid savannah, forest, forest gallery	Extrinsic
Tsetse distribution range	Number of countries where the taxa is found	Extrinsic
Geographic location of the survey	West African savannah, West African forest	Extrinsic
Bloodmeal preferences	Bovidae and suidae, bovidae mainly, human and other available hosts	Extrinsic

* Intrinsic or extrinsic to the trypanosome–tsetse association.

preferences, the taxa included in the present study represent 3 of the 5 distinct feeding groups of tsetse (Weitz, 1963; Clausen et al., 1998).

A random number variable was inserted in the database to represent a factor that had initially no influence upon the extent of trypanosome occurrence. A low contribution of the random variable would indicate little effect of chance on the infection, whereas a high value would indicate a large effect of chance (Ball et al., 2000; de Garine-Wichatitsky et al., 1999).

Analyses

Artificial neural networks (ANNs) were used to model both intrinsic and extrinsic parameters of trypanosome occurrence in tsetse. These models may combine a set of source variables (e.g., intrinsic and extrinsic) to predict an effect (Craig et al., 1999)—in this case, the likelihood of the presence of a given trypanosome occurring in tsetse midguts. In addition, ANN methods do not require a linear relationship between variables and so may be better suited to model nonlinear phenomena. ANNs also differ from general linearized models, e.g., logistic regression, in the way that the relationships between independent parameters and the predictor variables are estimated by an iterative trial-and-error procedure (Lek and Guégan, 1999).

The back-propagation algorithm for training the database with a typical 3-layer feed-forward (n-3–4) network (Guégan et al., 2000) was used; that is, n input neurons corresponding to the n independent parameters introduced into the model, 3 hidden neurons determined as the optimal configuration to obtain a best compromise between bias and variance, and 4 output neurons for the presence of each trypanosome taxonomic group. To assess the performance of the model, the total data set was partitioned into a first subset to train the model and a second subset to test its predictive power. The test used was a 'leave-one-out' or jackknife procedure (Efron 1983). This procedure leaves out a test set (1 tsetse fly $\times$ n inputs) from the training set (256–1 $\times$ n inputs), and this is repeated for each infected tsetse. The model deduced from the training set is then used to predict the presence or absence of a given trypanosome category in the test set. This was repeated for a maximum of 5,000 iterations for each tsetse fly.

First, the predictive value of each model was tested for assessing simultaneously the presence of each trypanosome taxonomic group in the midgut of the tsetse. The predictions obtained from the neural network model, e.g., predicted infected or predicted noninfected, were compared to the observed outcomes (e.g., infected, which are the true positives, and noninfected, which are the true negatives), and a percentage of 'good classification' was obtained. Second, and only when the model was shown to be useful, the contribution of each variable to the total variance of the trypanosome occurrence response was calculated by repeating 10 simulations of the same test. This test is based on Goh's (1995) algorithm, which allows discrimination of the effect of each independent parameter on the presence or absence of an event. This provides a way to obtain mean contribution values and confidence values around the mean for each predictor entered in the model (Lek and Guégan, 1999).

An association test using a correlation coefficient for binary data (Janson and Vegelius, 1981) was calculated to see whether associations between trypanosome groups were observed more frequently than by chance. In this test, all cases where the 2 trypanosomes occurred together were taken into account. All statistical analyses were performed with MatLab 5.0 for Macintosh software.

TABLE II. Taxonomic composition of the different coinfections identified in the midgut of the tsetse. *Tcs, Trypanosoma congolense* savannah type; *Tcf, T. congolense* forest type; *Ts, T. simiae*; *Tb, T. brucei.*

Type of multiple infection	No. of occurrences
Tcs/*Tcf*	53 (65%)
Tcs/*Tcf*/*Tb*	12 (15%)
Tcs/*Tcf*/*Ts*	1 (1%)
Tcs/*Tb*	5 (6%)
Tcs/*Ts*	3 (4%)
Tcf/*Ts*	3 (4%)
Tcf/*Tb*	4 (5%)
Total	81

RESULTS

Identity of the trypanosomes found in the midgut of tsetse

The most prevalent trypanosome subgroup detected by PCR was *T. congolense* savannah type (148 occurrences), followed by *T. congolense* riverine-forest type (116 occurrences), *T. simiae* (54 occurrences), and *T. brucei* (32 occurrences). The total number of identified trypanosome infections (350) was greater than the number of infected tsetse (256) because of multiple infections. Among the 256 infections, 175 were due to a single trypanosome group, 68 to coinfections by 2 trypanosome groups, and 13 to coinfections involving 3 trypanosome groups. Among the mixed infections, the trypanosome associations most frequently found together were savannah and riverine-forest types of *T. congolense* (65%), followed by a 3-way association involving these 2 groups with *T. brucei* (15%) (Table II).

Association tests between trypanosome groups yielded significant positive results for the savannah and riverine-forest types of *T. congolense* (Table IIIA; $P < 0.05$). None of the other association tests was significant (see Table IIIB for example). Applying the same test, but taking into account all the dissected tsetse (even the uninfected ones), the test became highly significant ($P < 0.0001$).

Prediction of presence of trypanosomes

Based on information entered into the model, the percentage of good classification scores were as follows: 60 and 88% for *T. congolense* savannah and riverine-forest types, respectively,

TABLE III. Association tests (correlation coefficient and corresponding chi-square statistics for binary data) illustrating the more represented trypanosome pairwise associations in the data set.

A. *Trypanosoma congolense* savannah type versus *T. congolense* riverine-forest type.

		T. congolense savannah type		
		Present	Absent	Total
T. congolense riverine-forest type	Present	66	50	116
	Absent	82	58	140
	Total	148	108	256
Chi square	6.223	$P < 0.05$		

B. *Trypanosoma congolense* savannah versus *T. brucei.*

		T. congolense savannah type		
		Present	Absent	Total
T. brucei	Present	17	15	32
	Absent	131	93	226
	Total	148	108	256
Chi square	1.505	$P > 0.05$		

87% for *T. brucei,* and 79% for *T. simiae.* However, negative scores, e.g., predicted uninfected/observed uninfected, varied greatly between 13 and 100% (Table IV). Positive scores (predicted infected/observed infected) also varied between 0 and 95%.

The best scores were obtained for the *T. congolense* riverine-forest type, with good classification scores for positive (90%) and true (87%) cases. For *T. congolense* savannah type, the classification was better able to detect its presence (95%) than its absence (13%). This could indicate that there was a bias in modeling capacity to detect the absence of *T. congolense* savannah type. Although good classification scores were obtained for *T. simiae* and *T. brucei,* their presence could not be predicted at all because of the low number of tsetse infected by these trypanosomes.

Contribution of explanatory variables to prediction

The contribution of each variable to the model for the 2 groups of *T. congolense* was calculated based on the best value obtained to model their presence. It should be stated here that, after a first look at the whole database, it appeared that some variables showed complete collinearity. For example, *G. p. palpalis* was the only tsetse taxon in the forest habitat, so these 2 variables were merged. The same was done for *G. morsitans submorsitans* and tsetse feeding on bovidae, as well as *G. longipalpis* and tsetse feeding on bovidae and suidae.

For savannah-type *T. congolense,* the results suggest that rather than only 1 or 2 variables, it is the simultaneous confounding influence of several independent variables that accounted for the presence of this trypanosome in the midgut of the tsetse analyzed. No variable contributed more than 7% to the total classification score, and the ''random'' variable introduced as an independent variable explained the presence of the savannah-type *T. congolense* as much as other independent parameters.

Alternatively, the presence of *T. congolense* riverine-forest type was best explained by the variable *Tcs* (i.e., the presence of *T. congolense* savannah type in the midguts of the tsetse), although its role was not significantly different from other independent variables after 10 runs (Fig. 1).

TABLE IV. Percentage of good total classification scores for each trypanosome taxonomic group, including positive (predicted infected/observed infected) and negative (predicted uninfected/observed uninfected) scores after 5,000 iterations.

	Good classification scores (%)	% Positive	% Negative
Trypanosoma congolense savannah type	60.7	95	13
T. congolense riverine-forest type	88.7	87	90
T. simiae	78.9	0	100
T. brucei	87.5	0	100

DISCUSSION

In the present study, the factors affecting the presence of trypanosomes in the midgut of tsetse were analyzed simultaneously in order to find the most significant. This approach has already been used in other host–parasite systems, e.g., to explain the prevalence of avian hemoparasites (Tella et al., 1999). To our knowledge, this is the first time it has been used in tsetse–trypanosome associations.

The analysis was made possible by using the PCR technique, which allows accurate identification of trypanosome taxa at the specific and intraspecific levels. PCR reactions were applied only on infected midguts (diagnosed using microscopy). Subsequently, only PCR positive results on these infected midguts were incorporated into the data set. Together with the assumption that DNA is rapidly degraded in the midgut (McNamara et al., 1995), this allows us to hypothesize that the trypanosomes identified represented established infections.

As a first attempt to predict the occurrence of trypanosomes in tsetse midguts, it was found more interesting to predict the presence of the trypanosomes than their absence, because the latter could be due to confounding factors (e.g., too few cases), the presence of undetected trypanosomes, or the lack of adequate variables.

The fact that the presence of *T. congolense* savannah and riverine-forest types could be predicted by the model suggests that the model incorporated some of the important variables influencing the establishment of a trypanosome infection in the vector. The model could be improved by including other factors that have not yet been incorporated in field studies (e.g., precise local climatic conditions, local habitat of the tsetse, availability of hosts, and intrinsic factors such as the presence of endosymbionts). The model could also benefit from replacing the discrete categorical variables by continuous ones, which would allow more powerful analyses. For example, values of normalized difference vegetation indices (NDVIs) provided by meteorological satellites could be used as climatic factors because they have been shown to be good predictors of the presence of tsetse in West Africa (Rogers and Randolph, 1991; Rogers et

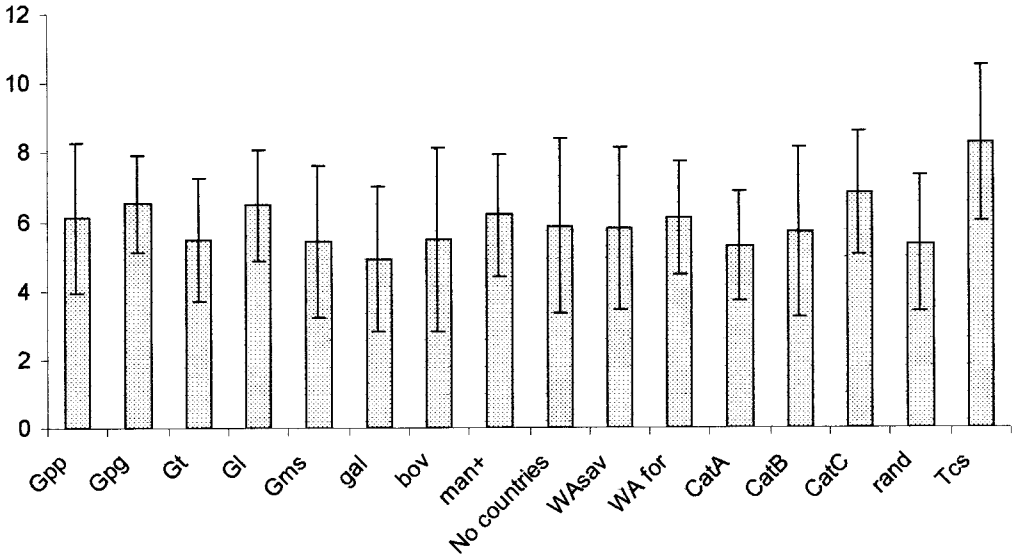

FIGURE 1. Histogram illustrating the contribution of different explanatory variables for the presence of *Trypanosoma congolense* riverine-forest type. Each bar gives the mean percentage of contribution for each explanatory variable with its corresponding 95% confidence interval after 10 runs. Legend: *Gpp, Gpg, Gt, Gl, Gms* are the 5 tsetse taxa investigated (see Table I); *gal,* forest gallery habitat; *bov,* tsetse preferentially feeding on bovids; *man+,* tsetse feeding preferentially on human and other available hosts; *no countries,* distribution range of tsetse taxa; *WAsav,* survey conducted in West African savannah zone; *WAfor,* survey conducted in West African forest zone; *cat A, B, C,* phylogenetic position of the tsetse (see text Materials and Methods: Explanatory variables); *rand,* random variable; *Tcs, Trypanosoma congolense* savannah type. Not all the initially chosen variables are represented because some were removed to avoid multicolinearity (see Results).

al., 1996). It will be also of great interest to extend this analysis to the trypanosomes found in the proboscis of the tsetse because these will represent mature infections, whereas an infection in the midgut will not necessarily lead to a mature infection (Maudlin et al., 1991; Reifenberg, Cuisance et al., 1997; Welburn and Maudlin, 1999).

A significant association between the savannah and riverine-forest taxonomic groups of *T. congolense* was demonstrated. Furthermore, the results suggested that the occurrence of *T. congolense* savannah type ranked first among all the potential influential factors when attempting to explain the presence of *T. congolense* riverine-forest type, although the reverse did not apply. These 2 taxonomic groups of *T. congolense* were defined first by Young and Godfrey (1983) on the basis of genetic differences revealed by enzyme electrophoresis. Thus, the riverine-forest group constituted stocks originating from the humid coastal zones of West Africa, whereas the savannah group contained stocks isolated from drier areas. Since then, the extensive use of PCR and DNA probes on naturally infected tsetse has shown that 'savannah' trypanosomes may be found in 'forest' tsetse (McNamara et al., 1995; Masiga et al., 1996; Morlais et al., 1998), as well as the reverse, i.e., 'forest' trypanosomes in 'savannah' tsetse (Lefrançois et al., 1999). A hypothetical representation of the interactions between these tsetse and the 2 types of *T. congolense* is presented in Figure 2. In several studies dealing with PCR identification of trypanosomes in tsetse, the riverine-forest type was rarely found alone (Solano et al., 1995). One exception was in Cameroon, in central humid Africa, where this group was the most prevalent in tsetse (Morlais et al., 1998). However, in Zimbabwe in a sample of more than 3,000 *G. pallidipes,* the riverine-forest type was never found without the savannah type of *T. congolense* in the same tsetse (Woolhouse et al., 1996). This latter result strengthens the observation that the 2 types of *T. congolense* were significantly associated in tsetse and that *T. congolense* savannah type appeared the most important variable accounting for the presence of the riverine-forest type.

Within the species *T. congolense,* the savannah type is the most prevalent in cattle in West Africa, whereas the riverine-forest type is very rare in cattle but more often present in domestic suids and small ruminants (Reifenberg, Solano et al., 1997; Lefrançois et al., 1998; Solano et al., 1999).

The riverine-forest type of *T. congolense* appears to originate from a forest biotope, seems to be poorly transmitted by various tsetse species (Reifenberg, Cuisance et al., 1997), and is rarely found alone in tsetse outside of its principal habitat. One has to reconcile these statements with the fact that it is frequently found in various tsetse taxa in immature and mature infections, together with its closely related savannah group of *T. congolense* in various ecological conditions (McNamara et al., 1995; Solano et al., 1995; Woolhouse et al., 1996).

A possible mechanism explaining its presence would be 'hitchhiking,' as proposed for other parasites (Thomas et al., 1998), e.g., the riverine-forest type of *T. congolense* would have a better chance to develop an infection and to be transmitted by tsetse already infected with the savannah type. To verify this hypothesis, further research must be conducted, focusing on experimental infections on tsetse sequentially infected with these 2 trypanosome types.

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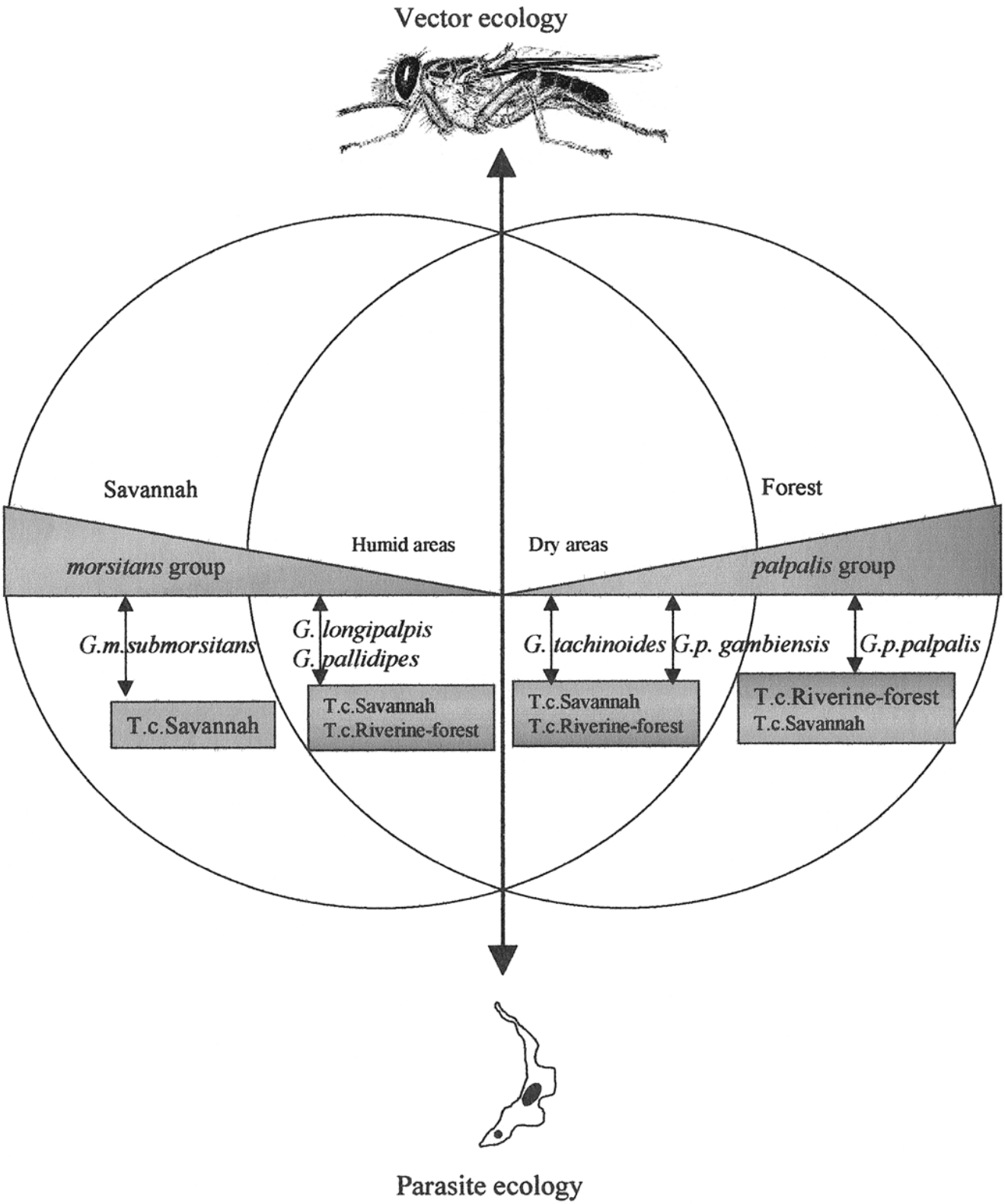

FIGURE 2. Hypothetical relationships between the 2 types of *Trypanosoma congolense* and their vectors, deduced from the results of field studies. Adapted from Reifenberg et al. (1996).

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Comparison of different DNA preparation protocols for PCR diagnosis of Human African Trypanosomosis in Côte d'Ivoire

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Abstract

During a medical survey the sleeping sickness focus in Bonon, Ivory Coast, PCR with *Trypanosoma brucei* specific primers (TBR 1–2 from Parasitology 99 (1989) 57) was tested on DNA derived from blood samples. DNA purification using a chelating resin was performed either on whole blood or on the buffy coat prepared in two different ways. The preparation based on whole blood performed better than those using the buffy-coat. Using this first method, the sensitivity was 100% on parasitologically confirmed patients, and the specificity was 92%. However, problems of reproducibility of the technique were pointed out, particularly on samples from serologically positive but apparently aparasitemic individuals. It is suggested that the PCR could help in the diagnosis of Human African Trypanosomosis, but the use of other primers should be investigated.

Keywords: Human African Trypanosomosis; Ivory Coast; PCR; Diagnosis; *Trypanosoma brucei gambiense*

1. Introduction

Human African Trypanosomosis (sleeping sickness) remains a significant public health problem in Sub-Saharan Africa. The impact of the disease is comparable to that of leishmanioses in terms of disability-adjusted life years lost (DALYS) (Molyneux, 1997). Since the disease is fatal if untreated and since some of the drugs used for treatment are very toxic, a reliable diagnosis is of paramount importance.

Mass screening of the population at risk is currently performed using serological tests in order to select individuals carrying trypanosome-specific antibodies, on which parasitological examinations are then carried out. The most commonly used serological tests in the field is the

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Card Agglutination Test for Trypanosomiasis (CATT), developed for detection of antibody to *Trypanosoma brucei gambiense* (Magnus et al., 1978). Because of its simplicity and low price, the CATT is a very useful tool for field application. The parasitological diagnosis of sleeping sickness is based on the detection of trypanosomes in lymph node aspirates, blood, or cerebrospinal fluid, but currently available tests have low sensitivity and are hampered by fluctuating parasitaemia (Truc et al., 1994). The existence of seropositive but parasitologically unconfirmed subjects (CATT + on whole blood and plasma, but negative using parasitological examination) may however have epidemiological implications, since some of them might actually harbor the parasite and thus contribute to the persistence of the foci.

PCR has been introduced for detection of trypanosome DNA in biological samples. In animal trypanosomosis, PCR has been used as a tool allowing precise identification of the infecting trypanosome taxa (Majiwa et al., 1994), or to detect trypanosomes in the blood (Katakura et al., 1997; Clausen et al., 1998; Solano et al., 1999). The technique has also been used on humans as a more sensitive alternative to parasite detection for primary diagnosis of *T.b. gambiense* infection (Enyaru et al., 1998; Kabiri et al., 1999; Simo et al., 1999; Kyambadde et al., 2000; Penchenier et al., 2000), or for stage determination of sleeping sickness by amplifying trypanosome DNA from the cerebrospinal fluid (Truc et al., 1999; Kyambadde et al., 2000). However, in all these studies, few efforts have been made to standardise the procedures and to comparatively evaluate either the sample preparation methods or the primer sets.

The objective of the present study was to compare simple methods for DNA preparation requiring minimal sample manipulation, which would allow PCR amplification from blood without the need for organic extraction of DNA. In addition, it was intended to evaluate whether PCR can be considered as a reliable diagnostic tool for human sleeping sickness, taking into account sensitivity, specificity, reproducibility and feasibility in field conditions.

2. Material and methods

2.1. Origin of samples

A medical survey was carried out in April–May 2000, around the town of Bonon, in Central West Ivory Coast (6°55′ N–6° W). A total of 13,900 people were screened using CATT on whole blood: among them 498 were positive (they were called 'suspects'). Of these 498 individuals, 170 were confirmed serologically positive by CATT on plasma (i.e. 'seropositive') and 76 (i.e. 'patients') were found infected with trypanosomes, using either the mini-anion exchange column (mAECT) or lymph node aspirates (49 and 27, respectively). The patients subsequently went to the Projet de Recherches Cliniques sur la Trypanosomiase (PRCT) in Daloa to receive treatment.

2.2. Preparation of blood and buffy-coat samples

Blood was collected in heparinized vacutainers during the medical survey and processed following three different protocols.

For comparison of preparations, 'true positives' will refer to PCR positive results on patients with demonstrated trypanosomes in the blood by mAECT only. 'False negatives' are negative PCR results on similar subjects. 'True negatives' are PCR negative results on CATT negative individuals (on whole blood). 'False positives' are PCR positive on the same samples.

In the first protocol, 1 ml of whole blood was dispensed in a 1.5 ml eppendorf tube (preparation 1).

The two other protocols used the buffy-coat instead of whole blood, because it has been reported that trypanosomes are concentrated at this level after centrifugation (Woo, 1970; Murray et al., 1977).

In preparation 2, after centrifugation of the vacutainer containing 5 ml of blood at $2000 \times g$ during 10 min, 50 μl of buffy-coat were put in a 0.5 ml eppendorf tube.

In preparation 3, 1 ml of blood was taken off from the vacutainer and put in a 1.5 ml eppendorf tube, whereafter the tube was centrifuged at

15,000 × *g* during 10 min; then 50 μl of buffy-coat were dispensed in a new 0.5 ml eppendorf tube.

The difference between preparations 2 and 3 relies on the assumption that in preparation 3, the buffy-coat would be easier to collect from a narrower tube than in preparation 2 thus reducing the risk of loss of trypanosome DNA.

After centrifugation and buffy-coat collection on the field, all these samples were kept in a coolbox during the day of collection, and put at − 20 °C when back in the laboratory.

On the 50 first patients who went to Daloa for treatment, we also wanted to assess which anticoagulant, i.e. EDTA or heparin, was the best for the PCR. One milliliter of blood was taken in an EDTA-coated tube and 1 ml in a heparinized tube, which were frozen immediately. On these samples, DNA extraction and PCR were performed according to the preparation 1 protocol described below.

2.3. DNA extraction procedures and PCR conditions

DNA extraction from preparation 1 was based on the protocol of Penchenier et al. (1996) and was modified by using a Chelex resin (Biorad) instead of the READYAMP (Promega), because the Chelex resin is much cheaper.

Preparations 2 and 3 were processed in the same way following a modified procedure of Walsh et al. (1991). Briefly, 100 μl of a 1% suspension of Chelex in pure water were added to the buffy-coat in the eppendorf tube, which was incubated at 56 °C for 1 h and at 95 °C for 30 min, whereafter it was centrifuged at 15,000 × *g* during 2 min. The supernatant constituted the DNA sample for PCR amplification.

The primers used were TBR 1–2 of Moser et al. (1989), which were reported to specifically amplify DNA of the subgenus *Trypanozoon* which comprises the species *Trypanosoma brucei*, and which have already been used to detect *T.b. gambiense* in humans (de Almeida, 1998; Enyaru et al., 1998; Truc et al., 1999).

A positive control (DNA of *T. brucei*), a first negative control (all the components of the PCR mix with milliQ water instead of DNA) and a second negative control (1% suspension of Chelex without any other product and processed in the same way as the samples) were run in each PCR reaction.

For PCR amplification, 5 μl of the Chelex-extracted supernatant was added to 25 μl of final reaction mixture containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 20 pmol primers and 0.5 U of Taq polymerase (Appligene): The DNA was first denatured at 95 °C for 1 min, then it was subjected to 35 cycles consisting in 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C, followed by a terminal elongation for 10 min at 72 °C. PCR products were visualized on an ultraviolet trans-illuminator after electrophoresis in a 2% agarose gel.

2.4. Strategy of analysis

First, the effect of heparin and EDTA was assessed by comparing the sensitivities obtained by PCR on paired samples from 50 of the 76 patients.

To determine the best method for DNA preparation, the sensitivities and specificities of PCR obtained through the three different preparations were compared on sets of samples from confirmed patients and from randomly chosen CATT negative individuals (Fig. 1). The sensitivity was classically calculated as the number of true positives divided by the number of patients detected by mAECt. The specificity was the number of true negatives divided by the number of CATT negative individuals (on whole blood), assuming that the latter were not infected. As sample sizes were relatively small, both sensitivity and specificity values are to be considered as indicative and should be confirmed on a larger population. Taking this point into account, confidence interval values were computed under the exact theoretical binomial distribution, allowing a more precise interpretation of these values (Dixon and Massey, 1969).

To measure the reproducibility of the PCR within each of these preparations (intra-preparation reproducibility), some samples were amplified by blind test a second time by the same person, a few days after the first amplification and κ values

Comparison	prep1	prep2	prep3
Sensitivity	26 T+	30 T+	14 T+
Specificity	49 CATT-	28 CATT-	ND
Intra preparation reproducibility	12 T+ 47 CATT- 34 suspect or seropositive	21 T+ 29 CATT- 40 suspect or seropositive	ND
Inter preparation comparison	12 T+, 28 CATT-, 19 suspect or seropositive		

Fig. 1. Sample analysis for comparisons of DNA preparations. T + : patient found infected with trypanosome using mAECt; CATT: individual negative using CATT on whole blood; ND: not done; suspect: individual positive using CATT on whole blood, negative using CATT on plasma (see also Section 2); seropositive: individual positive on both CATT on whole blood and plasma, but negative using mAECt.

for agreement were calculated. As proposed by Fleiss (1981), κ values lower than 0.40 reflect poor agreement, values between 0.40 and 0.75 reflect fair to good agreement and values above 0.75 indicate strong agreement.

The McNemar χ^2-test was used to compare the results obtained through different protocols performed (at blind) on the same individuals.

3. Results

3.1. Heparin versus EDTA

From 50 patients who were included in the protocol, 47 and 25 tested positive with PCR on blood taken, respectively into heparin and EDTA.

3.2. Sensitivity and specificity

In a first trial to compare the three preparations, the 14 first people who had been diagnosed as sleeping sickness patients, were tested. PCR gave 14 positive results with preparation 1, 13 positive results with preparation 2 and 11 positive results with preparation 3. We decided to continue the sensitivity and specificity tests only with preparations 1 and 2, because of the apparent low sensitivity of preparation 3.

Table 1
Sensitivity and specificity of the PCR using different sample preparations

PCR	Disease status		Total
	Infected	Uninfected	
(a) *Preparation 1*[a]			
Positive	26	4	30
Negative	0	45	45
Total	26	49	75
(b) *Preparation 2*[b]			
Positive	26	2	28
Negative	4	26	30
Total	30	28	58

'Infected' refers to a sample where trypanosomes were found, 'uninfected' is CATT negative on whole blood.

[a] Sensitivity: $26/26 = 100\%$; specificity: $45/(45+4) = 92\%$ with $CI_{95} = (0.84; 0.99)$.

[b] Sensitivity: $26/(26+4) = 87\%$ with $CI_{95} = (0.70; 0.97)$; Specificity: $26/(26+2) = 93\%$ with $CI_{95} = (0.74; 0.98)$.

Table 2
Within preparation reproducibility of the PCR, for two amplifications

			Total
(a) *With preparation 1*[a]			
Preparation 1 (1st)	+	−	
Preparation 1 (*2nd*)			
+	23	13	36
−	6	51	57
Total	29	64	93
(b) *With preparation 2*[b]			
Preparation 2 (1st)	+	−	
Preparation 2 (*2nd*)			
+	34	13	47
−	3	40	43
Total	37	53	90

[a] κ-test = 0.55.
[b] κ-test = 0.65.

Preparations 1 and 2 were compared regarding sensitivity and specificity (Table 1), respectively on 75 and on 58 of these 75 samples. Preparation 1 appeared the most sensitive, whereas the specificity was a little, but not significantly, higher with preparation 2. The best result appeared here with preparation 1, which yields 100% sensitivity and 92% specificity.

3.3. Intra-preparation reproducibility

Kappa values calculated on the results of respectively 93 and 90 samples (see Fig. 1 for the composition of samples) amplified twice through preparations 1 and 2, were 0.55 and 0.65, indicating 'fair to good agreement' for each preparation (see Table 2).

With preparation 1, from the 19 non-concordant results, 14 were suspect or seropositive individuals (see Fig. 1 for definition). On trypanosome-infected samples, 10 out of 12 gave concordant results, and on CATT negative samples, 44 out of 47 gave concordant results.

With preparation 2, from 16 non-concordant results, 14 were suspect or seropositive individuals. On infected samples, 20 out of 21 were concordant, and on CATT negative samples, 28 out of 29 were concordant.

3.4. Inter-preparation comparison

We compared the PCR results obtained through preparations 1 and 2 on 59 samples (Table 3). No significant differences were obtained when both preparations were performed on the same subjects ($P > 0.20$) indicating that none of these preparations led systematically to significantly more positive (or negative) results than the others. However, non-symmetric results appeared on 28.8% of the samples.

Moreover, when we checked the origin of the discordant results, we saw that, from 17 discordant results, again 11 originated from suspect or seropositive samples, four from CATT negative, and two from trypanosome infected samples.

4. Discussion

The presence in blood of components that inhibit PCR amplification has been a major problem associated with direct amplification of DNA in cell lysates (Higuchi, 1989). Therefore, many PCR methods involve organic extraction and ethanol precipitation to purify target DNA before amplification. While this yields DNA ready for amplification, the numerous manipulations required make organic extraction highly impractical for field use, and at the same time increase the risk of sample contamination with exogenous target DNA. To allow diagnosis of tropical diseases close to the field, PCR protocols should be simplified as much as possible without loss of

Table 3
Comparison of PCR results between preparations 1 and 2

Preparation 1	+	−	Total
Preparation 2			
+	14	11	25
−	6	28	34
Total	20	39	59

Between preparations 1 and 2. McNemar $\chi^2 = 1.47$ ns.

sensitivity and specificity. In a first attempt to identify an appropriate combination of blood sampling and PCR protocol for diagnosis of human sleeping sickness, we compared three different sample preparation procedures followed by the same PCR protocol, using the more often used TBR primer set of Moser et al. (1989). For DNA purification, we selected the Chelex procedure (based on Walsh et al., 1991) because of its simplicity and reported use (de Almeida et al., 1997; de Almeida, 1998; Solano et al., 1999; Truc et al., 1999).

In a first experiment, we looked at the effect of anticoagulants used for blood sampling, i.e. heparin and EDTA. It appeared clearly that heparin is more appropriate than EDTA when it is intended to perform PCR on the blood sample. Release of EDTA could provoke inhibition of the PCR. This confirms observations made by others who evaluated heparin, EDTA and citrate (Burckhardt, 1994).

On heparinized blood samples, we compared three DNA preparation protocols, one starting from whole blood and two starting from the buffy-coat, all being treated with a 1% aqueous Chelex suspension. Preparation 3 was only evaluated briefly, because it showed poor sensitivity on the first samples tested. Furthermore, it was the most time, labour and material consuming protocol, due to the transfer of the blood from the vacutainer to the eppendorf tube prior to centrifugation. The best compromise between sensitivity, specificity and simplicity was obtained with preparation 1, which showed 100% sensitivity on trypanosome infected patients detected during a medical survey and 92% specificity. It should be noticed here that, as we wanted the true positives to be positive with 100% certainty, we limited the sensitivity evaluation to samples that were taken from patients found infected with trypanosomes using the mAECT (26 for preparation 1, 30 for preparation 2). Further, we also tested the two preparations on patients who were found infected with trypanosomes by lymph node puncture (data not shown). Combining the two parasitological techniques (mAECT and lymph node puncture), the PCR results using the two preparations were the following: 100% sensitivity (43/43) with preparation 1, and 85.2% (46/54) with preparation 2, confirming the previous result.

At first instance, it seems surprising that with whole blood, the sensitivity appeared better than with the buffy-coat. Because of the fact that trypanosomes concentrate in a thin layer in the latter sample, we would have expected more trypanosome DNA to be present in the buffy-coat. An explanation for the lower sensitivity with the buffy-coat could be either the difficulty of properly taking the buffy-coat from the vacutainer tube, or the small volume taken (50 μl) taken for analysis, which would not allow enough DNA to be present for PCR amplification.

The 92% specificity of preparation 1 was due to four CATT-negative cases which appeared positive in PCR. We do not think that any mislabeling could account for this result. In addition, particular care was applied to avoid contamination by using two negative controls in each PCR. An infection with *T.b. brucei* might have occurred explaining a positive PCR result, but seems quite improbable. Although it has been shown that CATT may be negative in parasitologically confirmed patients (Dukes et al., 1992; Enyaru et al., 1998), this feature, to our knowledge has not yet been described in Ivory Coast. However, it cannot be excluded that some of these CATT false negatives could represent recently infected persons. It should be pointed out that PCR-positive results on CATT-negative samples have also been reported elsewhere using TBR primers (de Almeida, 1998; Penchenier et al., 2000). In the absence of more information, we consider these samples as inherent PCR false positives.

If the study had been stopped at this stage, the conclusion would have been that the technique used, i.e. PCR using TBR primers on whole blood treated with Chelex, is at least as satisfactory (100% sensitivity, 92% specificity) as other PCRs for sleeping sickness diagnosis (see Kabiri et al., 1999).

However, reproducibility was tested for each preparation and showed important discrepancies. On patients either 'suspect' or 'seropositive', the intra- or inter-preparation reproducibility appeared poor for both preparations. The CATT positive, but aparasitaemic subjects could repre-

sent either false CATT positives (but this cannot account for poor reproducibility), or false parasitological negatives with low parasitaemia. In this latter case, an explanation could be that very low amounts of DNA would induce a random possibility of taking sequences to be amplified. However, on patients on one hand, and on non-infected samples (i.e. CATT negative) on the other hand, the two preparations gave satisfactory, although not excellent, results (the number of concordant results on this type of samples was respectively 54 out of 59 for preparation 1 and 48 out of 50 for preparation 2).

In summary, the PCR using preparation 1 was the most sensitive, and gave satisfactory results on both trypanosome-infected and CATT-negative samples. On suspect samples (CATT positive on whole blood and/or plasma, but aparasitaemic) the results were completely inconsistent. Aiming at the use of PCR as a diagnostic tool for human sleeping sickness, it appears from the present study that a PCR positive result cannot be interpreted as a definite trypanosome infection. Taking into account the number of studies in which the PCR revealed new epidemiological features in trypanosomoses and appeared to be a promising diagnostic tool for several diseases (see Tang et al., 1998), we think that the unexplained results obtained in this study could be linked to the primers used. Other authors reported some unexplained results with these primers (de Almeida, 1998). Garcia et al. (2000), using these TBR primers in a longitudinal survey of seropositive individuals, even showed that in a cross-sectional study, positive PCR results might appear randomly in a population living in an endemic area. Our present results confirm the extreme complexity of seropositivity. In the near future, the samples used in the present work should be analyzed with other primers specific for *T. brucei s.l.* or for *T.b. gambiense*. It will be of particular interest to interpret a PCR positive result on suspects or seropositive samples.

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Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Côte d'Ivoire

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SUMMARY

In a sleeping sickness focus of Côte d'Ivoire, trypanosomes were characterized in humans, pigs and tsetse using various techniques. Out of 74 patients, all the 43 stocks isolated by KIVI (Kit for In Vitro Isolation) appeared to belong to only one zymodeme of *Trypanosoma brucei gambiense* group 1 (the major zymodeme Z3). The only stock isolated on rodents belonged to a different, new, zymodeme (Z50), of *T. b. gambiense* group 1. From 18 pigs sampled in the same locations as the patients, PCR showed a high proportion of mixed infections of *T. brucei s. l.* and *T. congolense* riverine-forest. Zymodemes of *T. brucei s. l.* from these pigs were different from those found in humans. From a total of 16 260 captured tsetse (*Glossina palpalis palpalis*), 1701 were dissected and 28% were found to be infected by trypanosomes. The most prevalent trypanosome was *T. congolense* riverine-forest type, followed by *T. vivax*, *T. brucei s. l.* and *T. congolense* savannah type, this latter being associated to the forest type of *T. congolense* in most cases. Mixed infections by 2 or 3 of these trypanosomes were also found. Use of a microsatellite marker allowed us to distinguish *T. b. gambiense* group 1 in some of the mature infections in tsetse. Differences in infection rates and in trypanosome genotypes according to the host might indicate that the pig may not be an active animal reservoir for humans in this focus.

Key words: sleeping sickness, Côte d'Ivoire, *Trypanosoma*, tsetse, microsatellite, animal reservoir, mixed infection.

INTRODUCTION

The protozoan parasite *Trypanosoma* (*Trypanozoon*) *brucei* is the aetiological agent of Human African Trypanosomosis (HAT, or sleeping sickness) in man, and nagana in animals. The human disease is a major public health problem in Africa (WHO, 1998), and the economic impact of the animal disease is a severe constraint for the development of this region. In addition to *Trypanozoon*, 2 other subgenuses are involved in nagana, *Nannomonas* (*T. congolense*) and *Duttonnella* (*T. vivax*), all being transmitted to the mammalian host by an infected tsetse during its bloodmeal.

In West Africa, the main vector is *Glossina palpalis*, and the pathogenic trypanosome for humans is *T. b. gambiense*. A major problem in the epidemiology of human disease is that *T. b. gambiense* is hardly differentiable from the 'animal' trypanosome *T. b. brucei*. Moreover *T. b. gambiense* has been found so scarcely in tsetse that our knowledge of transmission still suffers many gaps (Frezil & Cuisance, 1994), including the importance of an animal reservoir for the gambiense disease (Van Hoof, Henrard & Peel, 1937; Gibson *et al.* 1978; Mehlitz *et al.* 1982). In addition, controversy has occurred on the subspecies classification of *T. brucei s. l.* The only group which can be considered as a taxonomic unit is the one defined as *T. b. gambiense* group 1 (Gibson, 1986), which represents 80% of all West and Central African human isolates. Other trypanosome strains isolated from humans in West and Central Africa which did not belong to this group were classified in a heterogeneous *T. b. gambiense* group 2, or Bouafle group (Godfrey *et al.* 1990).

The development of molecular techniques applied to trypanosomosis, like DNA probes or PCR (Kukla *et al.* 1987; Moser *et al.* 1989; Majiwa *et al.* 1993) brought new insights on the epidemiology of the disease, first on animal trypanosomes with the discovery of many more multiple infections of different trypanosome groups in tsetse than previously suspected (Majiwa *et al.* 1994; Solano *et al.* 1995; Masiga *et al.* 1996). In the human disease, molecular studies to identify trypanosomes in tsetse and mammalian host remain scarce (but see McNamara *et al.* 1995; Morlais *et al.* 1998), certainly due to the lack of molecular markers able to distinguish 'human' and 'non human' trypanosomes within

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T. brucei s. l., although markers have been found in *T. b. rhodesiense* that can do this differentiation (Welburn *et al.* 2001; Radwanska *et al.* 2002). Here, in order to better understand tsetse/trypanosomes relations, we tried to characterize, by parasitological techniques and recently developed molecular markers, the trypanosomes found in the same place at the same time in tsetse, human host (patients), and one of the most suspected of potential animal reservoirs, the pig.

MATERIALS AND METHODS

The HAT focus of Bonon is located in Central West Côte d'Ivoire (6°55′N–6°W). Between 2000 and 2001, a medical survey was done on humans, followed by a survey on pigs located where the patients had been diagnosed. An entomological survey was also implemented at each season of the year in the area (see below).

Survey on humans and pigs; trypanosome isolation and molecular techniques

An exhaustive medical survey was implemented by the National Control Program of HAT to detect HAT cases, preceded by an exhaustive individual census of the entire population of the area (Solano *et al.* 2003). Sleeping sickness cases were detected by a classical procedure involving CATT test on blood and plasma (Card Agglutination Test for Trypanosomosis, Magnus *et al.* 1978), followed by mini-Anion Exchange Centrifugation Technique (mAECT, Lumsden *et al.* 1979), or lymph node examination when adenopathy was present. From each patient, 5 ml of blood were inoculated in a KIVI kit for trypanosome isolation (Aerts *et al.* 1992), followed by multiplication in axenic culture medium (Cunningham, 1977) according to the protocol described by Truc *et al.* (1992). The pellets of parasites were conserved in liquid nitrogen until use.

From a subsample of 16 of these patients (the first 16 who gave their consent), the blood was also inoculated in mice for another procedure of trypanosome isolation (mice isolation, MI). In detail, 0·5 ml of blood, from a heparinized tube, was injected intraperitoneally into 2 mice. The mice were inoculated with cyclophosphamide (Endoxan, 300 mg/kg) 2 days after blood injection, and then every 5 days. The mice were followed up every 3 days, from the 6th day after inoculation for a period of 2 months, by microscopical examination of tail blood. When parasitaemia became high (10^8 trypanosomes/ml), blood from the mouse was inoculated in rats. When the parasitaemia in the rat reached 10^8 trypanosomes/ml, a cardiac puncture was performed, and trypanosomes from the blood were filtered by using a macro-anion exchange column. The pellets of parasites were conserved into liquid nitrogen until required.

Blood was also taken from 18 pigs from the medical survey area. For each pig blood sample, parasitological diagnosis was performed by microscopical examination of the buffy coat (HCT, Murray *et al.* 1977), and each blood sample was inoculated both to KIVI and to mice for trypanosome isolation as described above.

MultiLocus Enzyme Electrophoresis (MLEE) was implemented to identify trypanosome zymodemes in humans and pigs. Proteins were extracted from the pellets of parasites previously grown by KIVI and MI according to the method of Truc, Mathieu-Daude & Tibayrenc (1991). Stocks were characterized by MLEE on cellulose acetate plates (Helena®) and 10 enzymatic systems representing 13 loci were revealed: ALAT (EC 2.6.1.2), GOT (EC 2.6.1.1), Nhi (EC 3.2.2.1), Nhd (EC 3.2.2.1), ME (EC 1.1.1.40), PEP-2 (EC 3.4.11), MDH (EC 1.1.1.37), IDH (1.1.1.42), PGM (EC 2.7.5.1), SOD (EC 1.15.1.1) (see Jamonneau *et al.* 2002 for details). For the analysis procedure, a UPGMA dendrogram was built starting with the Jaccard genetic distances (d, Jaccard, 1908) calculated from MLEE results to determine the relationships between stocks (Sneath & Sokal, 1973). Reference stocks of *T. b. gambiense* group 1 and Bouafle group were included, as well as stocks of *T. congolense*-like groups for the UPGMA comparison.

PCR with satellite and microsatellite markers was implemented on a subsample of 10 pigs to identify the trypanosome species/group which had been detected. A simple 1% Chelex treatment derived from the method of Walsh, Metzger & Higuchi (1991) was used to obtain DNA for PCR reactions (see Solano *et al.* 1999 for details). The following primers were used: TBR1-2 (Moser *et al.* 1989), specific for *T. brucei s. l.*, TCF 1-2 (Masiga *et al.* 1992), specific for *T. congolense* West African Riverine Forest (*T. congolense* F), TCS 1-2 (Majiwa *et al.* 1993), specific for *T. congolense* Savannah (*T. congolense* S). *T. vivax* primers were not used in pigs because of known refractoriness of the pig for this trypanosome (Kageruka, 1987). In addition, on all the trypanosome pellets (from humans and pigs grown by KIVI and MI), another primer pair was used: TRBPA 1-2, the product of which, a 149 bp fragment, has been reported to be specific for *T. b. gambiense* group 1 (Herder *et al.* 2002; Truc *et al.* 2002). A negative control was included at each chelex procedure and within each PCR reaction.

Entomological survey, tsetse dissection, sample collection

Following the medical survey, the key sites (home, water supply points, working places) of the dwellings of each patient was recorded by use of a Global

Positioning System (GPS) and subsequently 320 Vavoua traps (Laveissière & Grébaut, 1990) were settled in the area frequented by the patients. The captures were done in November 2000 (end of rainy season), January 2001 (cold dry season), April 2001 (end of hot dry season), and July 2001 (beginning of rainy season). Each trap remained for 4 days, with cages changed daily, and tsetse count, sex ratio, and dissection done daily. For the dissection, the mouthparts were first removed to prevent contamination from the midgut, and dissecting instruments were cleaned between each organ dissection with sodium hypochlorite, followed by rinsing in sterile water. Each organ (mouthparts, salivary glands, midgut) was put into a separate Eppendorf tube containing 30 μl of sterile distilled water.

For the PCR, we used the same 1% Chelex procedure as described above, and the same PCR primers as for the pigs (TBR 1-2, TCS1-2, TCF 1-2, TRBPA 1-2). In addition, primers specific for *T. vivax* were also used (TV1-2, Masiga *et al*. 1992).

RESULTS

Humans

Out of 15 227 people who came to the survey, 74 patients were found to have HAT, which gave a prevalence of 0·48% (0·47-CI_{95}-0·49).

From each of the 74 KIVI inoculated, 43 (58·1%) were successfully grown and trypanosomes were isolated. They all were shown to belong to the major zymodeme 3 of *T. b. gambiense* group 1 by MLEE (as Sique reference stock, see Fig. 1). From the 16 human stocks inoculated in mice, only 1 was successfully isolated (1 Human/MI). It was shown to have a different genotype (zymodeme 50) from all those isolated by KIVI. Using the diagnostic microsatellite marker TRBPA 1-2, the 149 bp band specific for *T. b. gambiense* group 1 was observed in all these samples, whatever the isolation method.

Pigs

By HCT, 4 pigs were found to be infected with trypanosomes. Out of 18 pigs, 13 *T. brucei* stocks were isolated by KIVI (72%), from which 8 were also isolated by MI (see Table 1). By PCR using satellite sequences on a subsample of 10 pigs, mixed infections involving *T. brucei s. l.* and *T. congolense* F were found in 9 pigs. In the 10th pig only *T. brucei s. l.* was found. No *T. congolense* S was detected in any of the pigs. All the *T. congolense* F which were identified by PCR were no longer present after isolation by KIVI or MI.

By MLEE, all the stocks isolated from pigs by KIVI and MI were different (but very close) to those isolated from humans and belonged to distinct *T. brucei s. l.* zymodemes, namely Z44 to Z49 (see Fig. 1). Details concerning the reference stocks have been reported by Jamonneau *et al*. (2003), from which Fig. 1 was modified by adding the human stock isolated by MI (1 Human/MI). When a stock was isolated simultaneously by the two techniques from each single pig, 2 different zymodemes of *T. brucei* were observed (already published by Jamonneau *et al*. 2003). Using TRBPA1-2, the 149 bp band specific for *T. b. gambiense* group 1 was detected in 9 trypanosome stocks isolated by KIVI (see Table 1) and in 1 stock isolated by MI (sample no. 6).

Tsetse

Parasitological results. During the 4 tsetse surveys between 2000 and 2001, a total of 16260 tsetse belonging to *G. p. palpalis* were caught, giving an apparent density of 3·19 flies/trap/day. From these, 1701 flies could be dissected. The parasitological infection rate obtained by dissection followed by microscopical examination was 28·1% [26·1-CI_{95}-30·1] (i.e. 478 flies were seen harbouring trypanosomes in either the midgut, the salivary glands, or the mouthparts).

Only 1 tsetse individual was found to be infected by trypanosomes in the salivary glands, which gives a *T. brucei s. l.* infection rate of 0·06% as determined by dissection. From the infected tsetse, 52·9% were infected in the midgut only, 27% in the mouthparts only (presumably *T. vivax*), and 19·6% had a concomitant infection of the midgut and the mouthparts (presumably *T. congolense*).

PCR results on infected tsetse. Because of logistical constraints, only 382 out of the 478 infected flies could be analysed by PCR. For each infected tsetse, PCR was implemented on mouthparts, salivary glands, and midgut. From these, 143 infections were due to *T. congolense* F (37·4%) which was the most prevalent, followed by *T. vivax* (95 occurrences, 24·9%), *T. brucei s. l.* (52 occurrences, 13·6%), and *T. congolense* S (16 occurrences, 4·2%). A total of 143 tsetse (37·4%) positive for trypanosomes by microscopy did not give any PCR signal (note that the total number of infections is more than 382 because of mixed infections).

Extended to all the dissected flies, the infection rates by species or groups of trypanosomes would give the following: 10·5% for *T. congolense* F, 6·9% for *T. vivax*, 3·8% for *T. brucei s. l.*, 1·1% for *T. congolense* S as determined by PCR (see Fig. 2).

From the 382 dissected tsetse analysed by PCR, 59 were infected by more than one trypanosome. The more frequent associations were: *T. congolense* F and *T. brucei s. l.* (16), and *T. congolense* F and *T. vivax* (16), followed by *T. congolense* F and *T. congolense* S (11), and *T. brucei s. l.* and *T. vivax* (8). *T. congolense* S and *T. vivax* was the less common association (1 case). In some tsetse, 3 different trypanosomes were observed (6 cases), even 4 (in 1 tsetse).

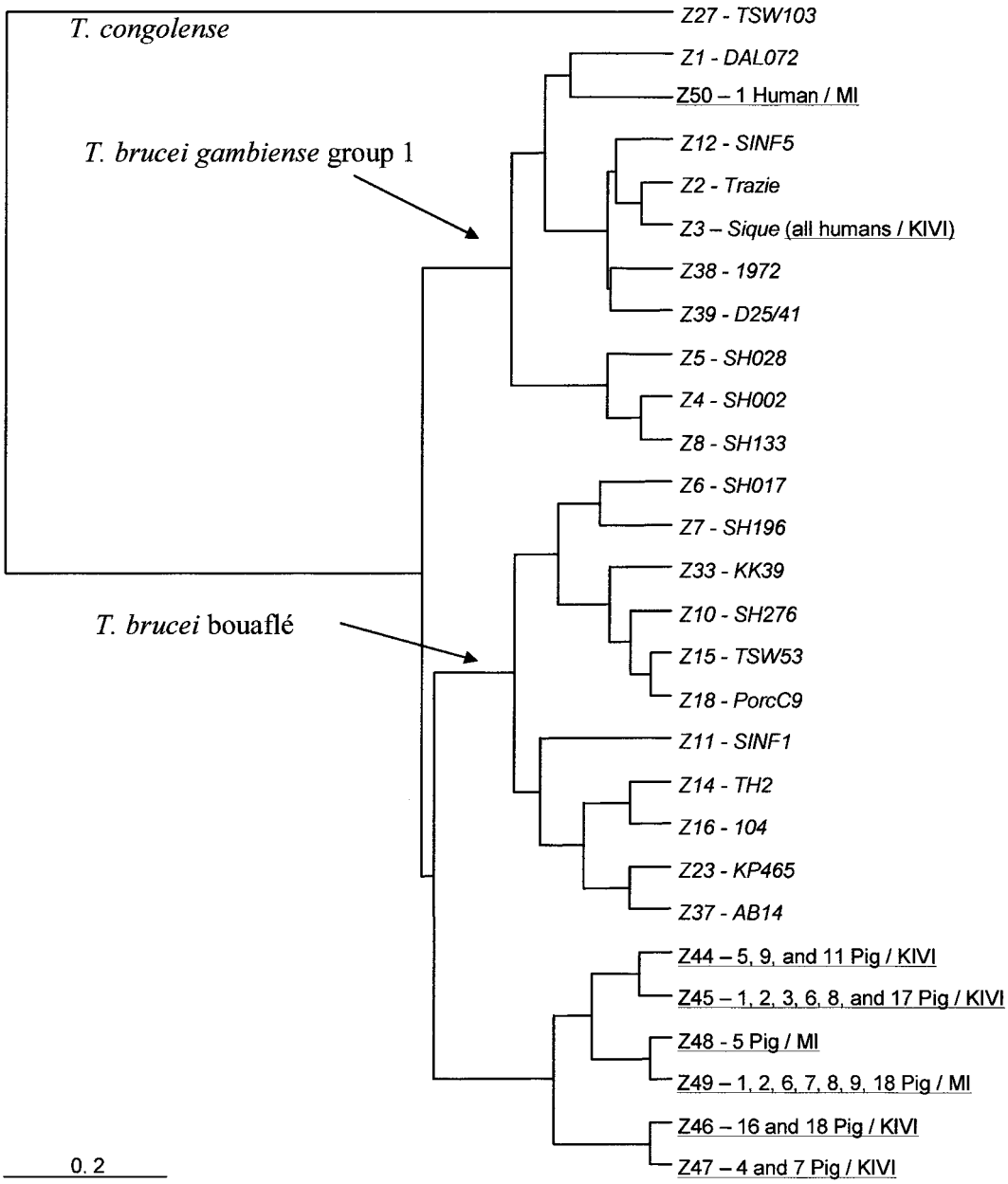

Fig. 1. Dendrogram showing genotypes obtained by MLEE on trypanosomes isolated from humans and pigs by mice inoculation and KIVI. The reference stocks are in italics and are preceded by their zymodeme number. The stocks obtained in the present study are underlined, are also preceded by their zymodeme number, and are followed by the host of origin (ex: sample Z48 19 MI/pig, is sample number 19, isolated from pig by mice inoculation, and belongs to zymodeme 48).

It has to be noted that of 16 infections with *T. congolense* S, it was found 15 times with another trypanosome. From 52 tsetse infected with *T. brucei s. l.*, 31 (59·6%) also harboured another trypanosome group.

Mature infections

For *T. congolense*, an infection was scored as mature if trypanosomes were found by PCR in either the proboscis and midgut concomitantly, or in the proboscis alone. In the latter case, we assume a disappearance of old *T. congolense* infections in the midgut, as previously described (Jordan, 1964).

For *T. congolense* F, 93 out of the 143 infections (65%) were mature. For *T. congolense* S, 12 out of 16 (75%) were mature. Hence, the mature infection rates for these trypanosomes in this study are: 6·8% for *T. congolense* F, and 0·8% for *T. congolense* S (see Fig. 2). Among the 12 mature infections of *T. congolense* S, all were mixed with other trypanosomes: in 10 tsetse, it was associated with mature infections of *T. congolense* F (among which, one also with *T. brucei s. l.*, and one with *T. brucei s. l.* and *T. vivax*), in one tsetse it was with *T. vivax*, and in the latter with *T. brucei s. l.* and *T. vivax*.

For *T. brucei s. l.*, an infection was considered as mature if both salivary glands and another organ

Table 1. Results of trypanosome detection and isolation in pigs by various techniques

(From 18 pigs, the search for trypanosomes was done first on the field using a parasitological technique (HCT, 2nd column). Blood was taken and returned to the lab, PCR was implemented for various species/groups of trypanosomes (3rd column) on some of the pigs (N.D.: not done). Mice and KIVI (4th and 5th columns) were also inoculated on the field and culture performed in the lab for trypanosome stocks which grew (see text for details). PCR/TRBP was then implemented on isolated stocks to check for the 149 bp allele specific for *T. b. gambiense* group 1 (6th column).)

Sample no.	HCT on the field	PCR result on blood	mice isolation	KIVI isolation	(PCR/TRBPA) on KIVI stocks
1	N.D.	**Tb**; **Tcf**	**yes**	**yes**	**Tbg1**
2	Neg.	**Tb**; **Tcf**	**yes**	**yes**	**Neg.**
3	**T+**	**Tb**; **Tcf**	no	**yes**	**Tbg1**
4	Neg.	**Tb**; **Tcf**	no	**yes**	**Tbg1**
5	**T+**	**Tb**	**yes**	**yes**	**Tbg1**
6	**T+**	**Tb**; **Tcf**	**yes**	**yes**	**Tbg1**
7	Neg.	**Tb**; **Tcf**	**yes**	**yes**	**Neg.**
8	Neg.	**Tb**; **Tcf**	**yes**	**yes**	**Tbg1**
9	**T+**	**Tb**; **Tcf**	**yes**	**yes**	**Tbg1**
10	Neg.	**Tb**; **Tcf**	no	no	N.D.
11	Neg.	N.D.	no	**yes**	**Neg.**
12	Neg.	N.D.	no	no	N.D.
13	Neg.	N.D.	no	no	N.D.
14	Neg.	N.D.	no	no	N.D.
15	Neg.	N.D.	no	no	N.D.
16	Neg.	N.D.	no	**yes**	**Tbg1**
17	Neg.	N.D.	no	**yes**	**Tbg1**
18	Neg.	N.D.	**yes**	**yes**	**Neg.**

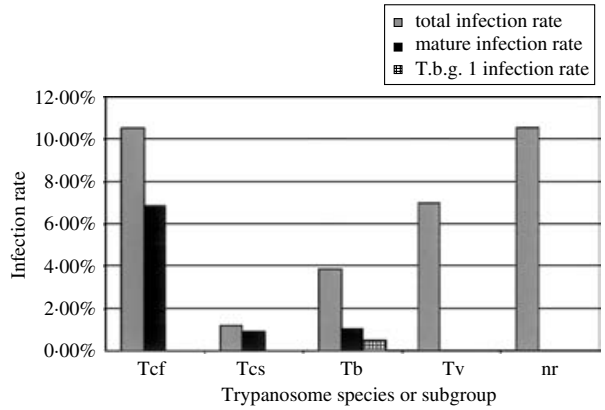

Fig. 2. PCR infection rates by species or groups of trypanosomes. Results obtained by PCR on the infected tsetse, extended to all dissected tsetse. Tcf: *Trypanosoma congolense* 'West African riverine forest' type; Tcs: *T. congolense* 'Savannah' type; Tb: *T. brucei s. l.*; Tv: *T. vivax*; nr: 'not recognized', i.e. infections detected by microscopy which did not give any amplification signal by PCR with the primers used. Tbg: *T. brucei gambiense* group 1. See text for the definition of 'mature infection rate', according to trypanosome species.

(proboscis and/or midgut) were found simultaneously positive by PCR. Of the 52 tsetse found infected with *T. brucei s. l.*, 14 had such mature infections. This would give a total mature infection rate of *T. brucei s. l.* of 1% (see Fig. 2). On these 14 tsetse having mature *T. brucei s. l.* infections, we used the *T. b. gambiense* group 1 specific primer set (TRBPA1-2, see Fig. 3). Seven of them showed the specific 149 bp band of *T. b. gambiense* group 1 at least in the salivary glands (Table 2). This would give a 0·5% infection rate of mature *T. b. gambiense* group 1 as determined by PCR.

Out of these 7 *T. b. gambiense* group 1 infections in the salivary glands, only one was a mixed infection involving another trypanosome (*T. congolense* F). Whereas of the 7 *T. brucei s. l.* infections that were not recognized as *gambiense*, 5 were mixed with other trypanosomes (see Table 2).

DISCUSSION

This study carried out in the HAT focus of Bonon in Côte d'Ivoire was performed to more clearly understand trypanosomes/tsetse/human and animal relations by characterizing trypanosomes found in the same place at the same time in these various potential or actual components in HAT epidemiology.

First, in humans, as expected using KIVI, only 1 zymodeme of *T. b. gambiense* group 1 was observed by MLEE, the predominant zymodeme Z3. Jamonneau *et al.* (2002) already reported this strong monomorphism, which was subsequently attributed to the selective nature of KIVI (Jamonneau *et al.* 2003). Here indeed, the only stock isolated from a patient by MI (Z50) was found to be genetically different by MLEE from all those isolated by KIVI (Z3), although both were shown to belong to *T. b. gambiense* group 1 by the TRBPA 1-2 marker. As isolation and culture stages probably select genotypes, the real genetic diversity of *T. b. gambiense* in

Table 2. PCR results on tsetse having mature *Trypanosoma brucei* infections

(See text for definition of mature *T. brucei* infections; + positive; − negative. For PCR results, i.e. with PCR using TBR1-2, TCF1-2, TCS1-2, TV1-2 primer pairs, positive = presence of the band at the expected size. For TRBPA1-2 primer pair, positive = presence of the 149 bp fragment specific for *T. b. gambiense* group 1. *T.c.f.*: *T. congolense* 'West African riverine forest' type; *T.c.s.*: *T. congolense* 'Savannah' type; *T.b.*: *T. brucei s. l.*; *T.v.*: *T. vivax*. *T.b.g.* group 1: *T. brucei gambiense* group 1. For instance, tsetse no. 3 is infected by *T. brucei s. l.*, *T. congolense* F and *T. vivax*. Tsetse no.6 is infected by *T. b. gambiense* group 1.)

Sample no.	Sex	PCR TBR1-2	PCR TCF1-2	PCR TCS1-2	PCR TV1-2	PCR TRBPA	Interpretation
1	F	+	−	−	−	−	*T.b.*
2	F	+	−	−	−	−	*T.b.*
3	F	+	+	−	+	−	*T.b./T.c.f./T.v.*
4	M	+	+	−	−	−	*T.b./T.c.f.*
5	F	+	+	−	−	−	*T.b./T.c.f.*
6	F	+	−	−	−	+	*T.b.g.* group 1
7	F	+	−	−	−	+	*T.b.g.* group 1
8	F	+	−	−	−	+	*T.b.g.* group 1
9	F	+	−	−	−	+	*T.b.g.* group 1
10	F	+	−	−	−	+	*T.b.g.* group 1
11	F	+	+	−	−	+	*T.b.g.* group 1/*T.c.f.*
12	F	+	−	−	−	+	*T.b.g.* group 1
13	F	+	−	−	−	−	*T.b.*
14	F	+	−	+	+	−	*T.b./T.c.s./T.v.*

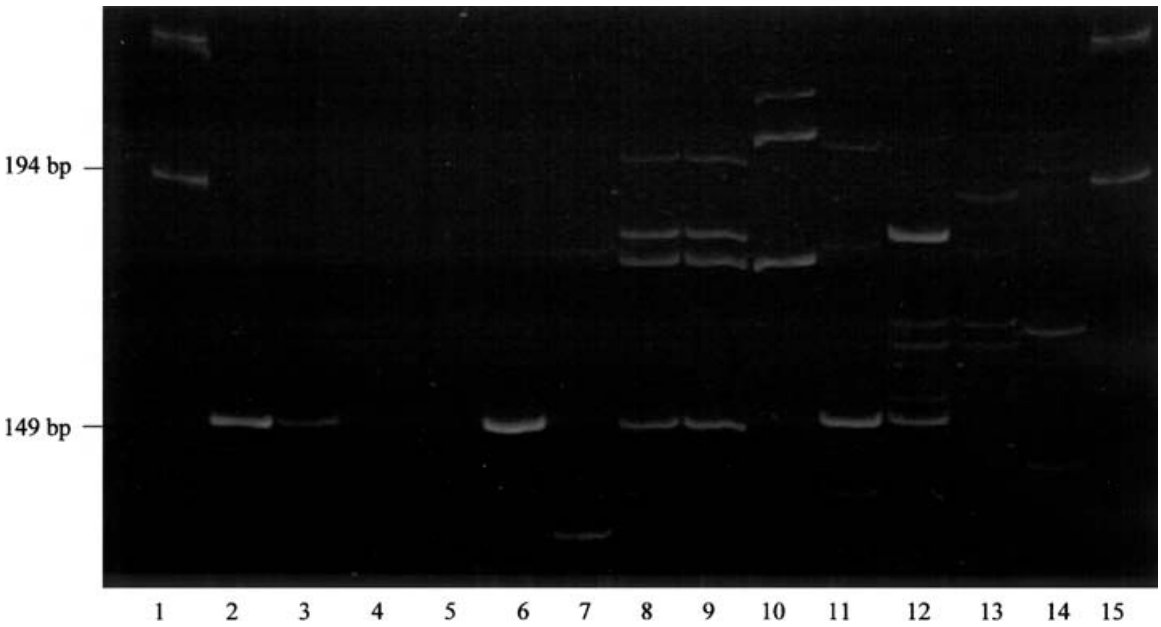

Fig. 3. Acrylamide gel with TRBPA showing the 149 bp fragment diagnostic of *Trypanosoma brucei gambiense* group 1 in reference stocks and in salivary glands and midguts from field-caught tsetse. Lanes 1 and 15: molecular marker (M4, Eurogentec), Lane 4: negative control, Lane 8: *Trypanosoma brucei gambiense* group 1 reference stock (Jua, Truc *et al*. 1991). Lane 9: *T. b. gambiense* group 1 reference stock (Peya, Truc *et al*. 1991). Lane 10: *T. b. brucei* reference stock (Stib215, Gibson *et al*. 1978). Lanes 2 and 3: salivary gland and midgut of a tsetse infected by *T. b. gambiense* group 1 in these two organs, Lanes 5, 6 and 7: salivary gland, midgut, and proboscis of a tsetse infected by *T. b. gambiense* group 1 in the midgut only, Lanes 11, 12 and 13: salivary gland, midgut, and proboscis of a tsetse infected by *T. b. gambiense* group 1 in the salivary glands and midgut, Lane14: midgut of a tsetse in which *T. b. gambiense* group 1 was not found.

humans must be higher than previously observed. For instance, multiple infections by different genotypes of *T. brucei s. l.* have recently been reported in patients (Truc *et al*. 2002; Jamonneau *et al*. 2004), as well as in tsetse (Letch, 1984). This strongly suggests that direct identification of trypanosomes within blood, without involving isolation and culture stages should be used in the future, provided that sensitive and specific molecular markers are available.

In our sample of 18 pigs, a high trypanosome prevalence was observed (72·2%), in accordance with previous studies (Mehlitz, 1986). The high

prevalence of mixed infections of *T. congolense* F and *T. brucei s. l.* in pigs also confirms previous results (Mehlitz, 1986; Noireau *et al.* 1986), with additional information here on the *T. congolense* molecular group. We also noted that on pigs infected with these two trypanosome species, following a culture step (with both KIVI and MI) *T. brucei s. l.* always outcompeted *T. congolense* F which systematically disappeared.

Jamonneau *et al.* (2003) recently showed by MLEE that zymodemes of *T. brucei s. l.* on a single pig were different according to the isolation method used (KIVI and MI). In the present work, in contrast to other studies (Gibson *et al.* 1978; Mehlitz *et al.* 1982), zymodemes found in pig by MLEE were all different (albeit very close) to those found in humans whatever the isolation method used. By PCR with TRBPA 1-2, the specific 149 bp fragment of *T. b. gambiense* group 1 appeared in some of the stocks, confirming the close relationship between stocks from pigs and from humans.

These results suggest a very high diversity of genotypes of trypanosomes at the intra- (e.g. within *T. brucei s. l.*) and inter-specific level (e.g. *T. brucei s. l.* and *T. congolense*) circulating in pigs in this HAT focus, the majority of them remaining uncharacterized. It appears also that various genotypes circulate in humans, but that the ones which were characterized differ from those found in pigs, although again many genotypes of *T. brucei s. l.* in humans are still unknown (all those not isolated by MI nor by KIVI).

In tsetse, trypanosome infection rates observed using microscopical examination were higher than generally reported in West Africa (Squire, 1954; Lefrançois *et al.* 1998), even in Côte d'Ivoire (Croft *et al.* 1984; Nekpeni *et al.* 1991). An important number of infections seen by microscopical examination could not be characterized by PCR, as already reported (Solano *et al.* 1995; Lefrançois *et al.* 1998; Morlais *et al.* 1998). This may be attributed to the fact that the range of primers used in this study could not identify all the trypanosomes known to develop in the tsetse gut, e.g. *T. simiae*, *T. varani*, Tsavo *T. congolense* (Hoare, 1972; Majiwa *et al.* 1993), and also to PCR inhibition due to elements present in the midgut (Ravel *et al.* 2004).

T. congolense F was the most prevalent trypanosome in *G. p. palpalis*, as expected in this transitional forest-savannah area around a peridomestic habitat (see also Morlais *et al.* 1998) where pigs are abundant, and also small ruminants in which *T. congolense* F can develop. The higher prevalence of *T. congolense* F in *G. p. palpalis*, and its common association with *T. brucei s. l.* in mixed infections strenghtens the idea that there may be a predominant pig/tsetse/pig cycle in this area, also suggested by the reported bloodmeal preferences of *G. p. palpalis* for the pig (Sané, Laveissiere & Meda, 2000).

T. congolense S was weakly present in *G. p. palpalis* and, when present, was in most of the cases, associated to its closely related *T. congolense* F. In Toumodi, which is located in the same country at the same latitude as Bonon but in a savannah area, the reverse was observed in *G. longipalpis*, i.e. few *T. congolense* F, but always associated with *T. congolense* S which was the most prevalent (Solano *et al.* 1995). It may be that some evolutive mechanisms of association between these 2 trypanosome groups exist that help them to succeed in their transmission cycle in adverse ecological conditions (Solano *et al.* 2001).

Looking at mature infections, with parasitological detection only 1 salivary gland was found infected with trypanosomes out of 1701 dissected tsetse, which has been classically reported (Hoare, 1972). The use of PCR permitted a higher detection rate for *T. brucei s. l.* mature infections (in 14 tsetse) (see also Ravel *et al.* 2003). In addition, using a primer pair specific for the detection of *T. b. gambiense* group 1, this latter was found in 7 of the 14 mature infections. Eventually, the mature *T. b. gambiense* group 1 infection rate in tsetse was 0·5%, which is very close to the human prevalence of the disease in this focus (0·48%).

The great number of mixed infections involving 2, 3 or even 4 trypanosome groups in a single tsetse confirms previous results (Masiga *et al.* 1996; Woolhouse *et al.* 1996; Lehane *et al.* 2000), and supports the idea that tsetse can be sequentially infected on the field, at least for *T. congolense* and *T. vivax* (Squire, 1954). This might be different for trypanosomes infecting humans, which would infect tsetse mostly at their first bloodmeal (Van Hoof, 1937; Maudlin & Welburn, 1994), thus explaining a lower infection rate in tsetse for these trypanosomes. In addition, we observed in the present study that from the 7 tsetse harbouring a mature infection with *T. b. gambiense* group 1, only one was infected with another trypanosome, which might mean that either an established *T. b. gambiense* infection will prevent, in most cases, other trypanosomes from reaching a mature stage (when the first bloodmeal is on an animal), or that a tsetse infected by *T. b. gambiense* on a human (which occurs not frequently) will feed afterwards more readily on a human. In both cases, it can be assumed that a man/tsetse/man cycle, although occurring much less frequently than a pig/tsetse/pig cycle in this area, can mostly explain by itself the close and low prevalences of *T. b. gambiense* in tsetse and humans in this focus. Then, looking at the high infection rate of *T. brucei s. l.* in pigs, it has to be deduced that most of these trypanosomes will probably not be transmitted to humans. In this study, this is strenghtened by the fact that zymodemes found in pigs were all found to be different from those found in humans, although some of them belonged to *T. b. gambiense* group 1. However, this latter point has to be taken with caution, given that most of the

genotypes circulating were not identified because of selection by culture.

Although being quite speculative, these scenarii would suggest that in the field in this area, there are few chances that a human could be infected by trypanosomes originating from pigs via a tsetse.

An interesting outcome of this study is the ability to identify *T. b. gambiense* group 1 trypanosomes in tsetse, although it is not known if the *T. b. gambiense* group 1 identified by TRBPA 1-2 in tsetse and in pigs are infective for humans. Another outcome is that the study was conducted in the different hosts of the pathogenic system at the same place and at the same time, and it shows a very high diversity and intense circulation of multiple different trypanosome genotypes of different species and subspecies among the different hosts. It also appears important to study the circulation of animal trypanosomes from which interactions with 'human' trypanosomes may help to understand the transmission of the human disease. We are aware that these results only involve this focus and might not be extended to others, and also that our sample of pigs was not sufficient to reach definitive conclusions. In the future, higher numbers and different species of animals should be used. Direct identification of trypanosomes will hopefully be performed without culture steps to avoid sampling bias, because many genotypes of *T. brucei* have never been isolated and could change the conclusions drawn so far.

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L'impact de la guerre sur l'evolution de la THA dans le centre-ouest de la cote d'ivoire

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Résumé

OBJECTIF Evaluer la situation de la maladie du sommeil dans le centre-ouest de la Côte d'Ivoire de 2000 à 2003, en tenant compte des évènements survenus depuis en septembre 2002.
MÉTHODES Enquête active réalisée par des équipes médicales et détection passive des cas.
RÉSULTATS Entre 2000 et 2003, 250 patients ont été diagnostiqués pour la maladie du sommeil. De prime abord la prévalence de la maladie du sommeil semblait avoir baissé depuis le début de la guerre. Mais cette baisse apparente était due à une faible couverture de la population. La participation dans l'enquête médicale était différente selon le groupe ethnique, reflétant les conflits entre les différentes communautés pour les terres. De tels conflits sont courants dans la zone mais ont été exacerbés par la guerre.
CONCLUSION L'évaluation de l'importance de la maladie du sommeil durant la guerre par enquête médicale seule est très difficile. Mais la détection de la maladie du sommeil par surveillance passive a augmenté.

mots clés Trypanosomiase Africaine Humaine, épidémiologie, côte d'Ivoire, guerre, troubles sociopolitiques

Introduction

La Trypanosomose Humaine Africaine (THA) ou maladie du sommeil transmise par la mouche tsé-tsé (ou glossine), est une maladie négligée car on la croyait éteinte depuis les années 1960. Ceci contribue probablement à expliquer en partie son actuelle résurgence, puisque l'OMS estime de 300000 à 500000 le nombre actuel de cas (WHO, 2001). Plus grave encore est la très faible proportion de personnes bénéficiant d'une surveillance (4 millions sur 55 millions exposées). Certains pays, notamment ceux où des troubles socio-politiques sont survenus, connaissent un dramatique retour à la situation qui prévalait dans les années 1920.

En Afrique de l'Ouest, c'est la forme dite chronique à *Trypanosoma brucei gambiense* qui prévaut, transmise principalement par l'espèce de tsé-tsé *Glossina palpalis*. En Côte d'Ivoire plus particulièrement, il est classiquement admis que c'est le développement des cultures de rente (café, cacao), permis par un apport massif de main d'œuvre immigrée qui a entraîné une transmission plus intense de la THA (Hervouët & Laveissière, 1987). La Côte d'Ivoire est classée comme pays hyper-endémique par l'OMS (OMS, 2001). Suite à un premier bilan fait sur la répartition géographique des trypanosomés en Côte d'Ivoire de 1993 à 2000 (Dje *et al.* 2002), l'objectif de ce travail est d'actualiser l'évolution de la THA dans le centre-ouest de la Côte d'Ivoire entre 2000 et 2003, en termes de nombre de malades dépistés et de personnes vues lors des prospections médicales actives, en mettant un accent particulier sur les évènements survenus depuis le 19 septembre 2002 et leurs conséquences possibles sur la THA.

Matériel et méthodes

Zones d'étude

En Côte d'Ivoire, les 2 centres de traitement de la THA actuellement opérationnels sont situés dans le centre-ouest à Daloa (Projet de Recherches Cliniques sur la THA, PRCT) et Bouaflé (District Sanitaire, DS). Les prospections médicales menées par les équipes mobiles concernent en priorité cette zone du centre-ouest, et plus précisément les régions de la Marahoué et du Fromager (Figure 1).

Les foyers prospectés dans ces régions sont:

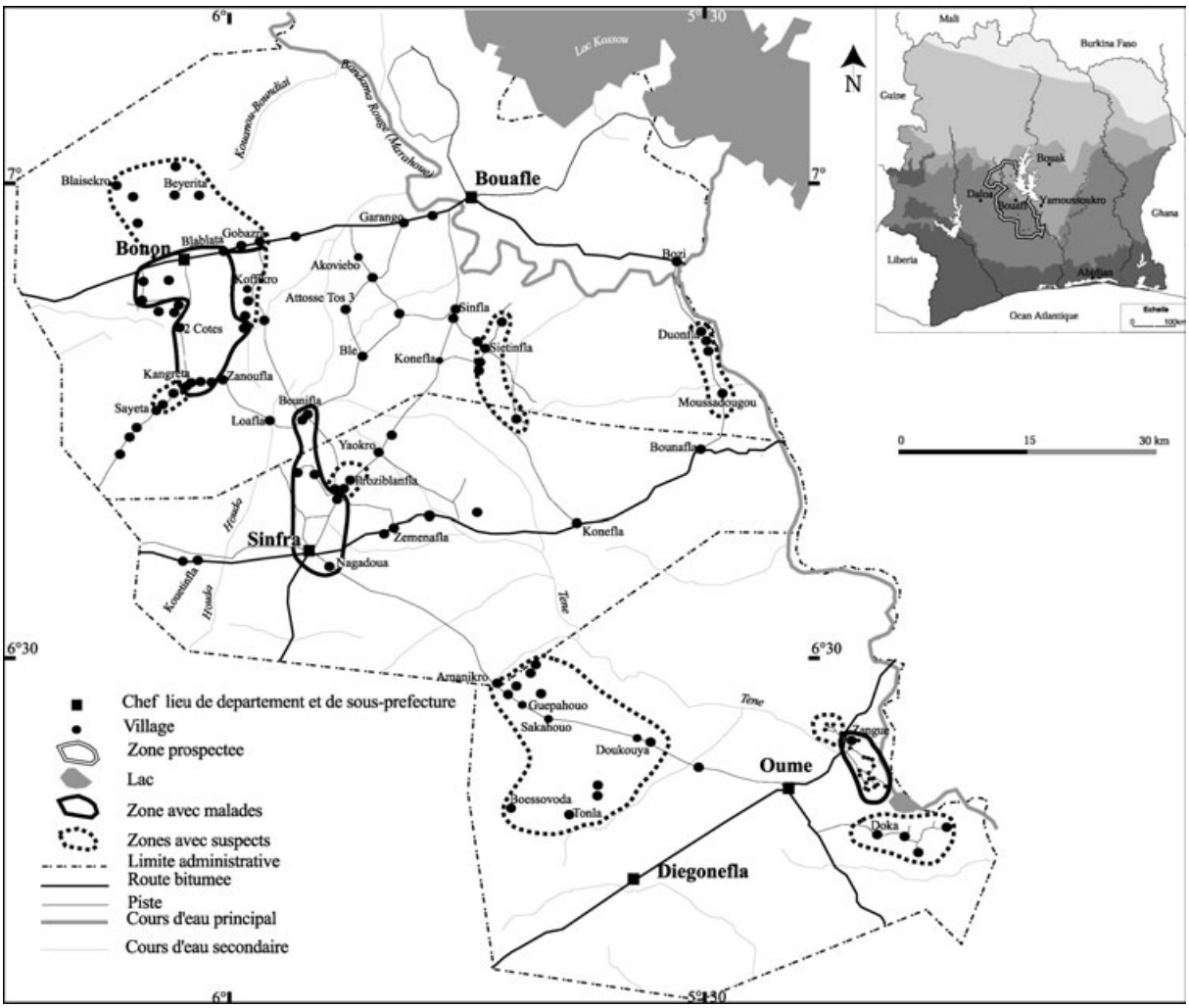

Figure 1 Situation de la THA dans le centre-ouest de la Côte d'Ivoire de 2000 à 2003.

- le foyer historique de Bouaflé, dont une zone a été vue en 2003.
- les foyers plus récents et actifs de Sinfra et Bonon. Ces deux foyers ont été régulièrement visités en totalité ou en partie en 2000 (Bonon seul), 2002 et 2003.
- la zone de Oumé, vue en 2001 et 2003.

De manière générale, la végétation dans ces zones est originellement la forêt mésophile qui ne subsiste qu'à l'état de quelques reliques forestières. Ces zones sont fortement anthropisées avec présence importante de cultures de rente (cacao, café principalement), et secondairement de cultures vivrières.

Populations d'étude

La population est hètérogène dans le centre-ouest avec une forte composante allogène ivoirienne et étrangère. Les autochtones sont Gouro et Gagou. La population allogène est importante composée d'Ivoiriens Baoulé, Sénoufo, Dioula, Lobi, et de ressortissants ouest africains, Burkinabés en majorité (Mossi, Lobi…), mais aussi Maliens, Guinéens, Béninois.

Ces populations vivent dans les villes, villages, hameaux et campements, et exercent essentiellement une activité agricole de rente ou des activités dérivées (commerce, etc.).

Depuis le 19 septembre 2002, date du début des évènements sociopolitiques en Côte d'Ivoire, ces populations, particulièrement celles du centre-ouest, ont accueilli de nombreux déplacés venus majoritairement de l'Ouest et du Nord du pays, tandis qu'un retour massif de ressortissants ouest-africains a eu lieu vers leurs pays d'origine.

Le dépistage actif

Nous avons pris en compte les résultats de tous les dépistages actifs par prospections médicales entre 2000 et 2003.

Avant la prospection, un important travail de sensibilisation est fait au près des chefs de villages, de hameaux, et des différentes communautés ethniques, religieuses, politiques et sociales, les informant de notre passage.

Des séances d'explication portant sur la maladie (mode de transmission, manifestations cliniques, complications, etc.) sont proposées aux populations en présence des chefs de communautés, des organisations de jeunes, etc. Cette information est capitale pour être sûr que les populations comprennent bien pour quel motif l'équipe se dèplace jusque chez eux, et ne puisse pas confondre avec d'autres évènements (politique, autre maladie, etc.), surtout en tenant compte de la situation actuelle en Côte d'Ivoire.

La méthode de diagnostic est celle classiquement utilisée en Afrique de l'Ouest, basée successivement sur le tri sérologique au CATT (Card Agglutination Test for Trypanosomiasis, Magnus *et al.* 1978) sur sang total, puis sur le plasma lorsque le premier CATT est positif. L'examen de confirmation parasitologique à la minicolonne (mAECT, Lumsden *et al.* 1979) est ensuite effectué chez les sujets positifs à ce dernier test. Par ailleurs, une ponction ganglionnaire avec recherche de trypanosomes entre lame et lamelle est effectuée chez tous les sujets porteurs d'adénopathies ayant un CATT positif au sang total.

Le dépistage passif dans les centres de traitement:

Nous avons pris en compte les résultats de tous les dépistages passifs effectués en consultation dans les centres de traitement (DS de Bouaflé et PRCT de Daloa). Une consultation des cahiers de registre des deux centres nous a permis de notifier tous ces cas.

La définition des cas et des séropositifs

La définition du cas est purement parasitologique par la mise en évidence du trypanosome dans le sang, le suc ganglionnaire ou le liquide céphalo-rachidien.

Les séropositifs sont ceux dont le CATT sur sang et sur plasma étaient positifs mais chez qui le parasite n'a pu être mis en évidence.

Analyse statistique des résultats:

L'analyse statistique des données a été effectuée sur Epi info version 6, 2000 et le test utilisé pour la comparaison des proportions et pourcentages est le Chi deux avec un risque α de première espèce de 5%.

Résultats

Prospections médicales actives

Au total, entre 2000 et 2003, 48 747 personnes ont été vues lors des prospections médicales actives (« équipes mobiles ») organisées conjointement par l'Institut Pierre Richet (IPR) de Bouaké et le PRCT de Daloa, sous la coordination du Programme National de Lutte contre la THA.

Sur le Tableau 1 figurent le nombre de personnes vues, le taux de présentation estimé, la prévalence et la sérophéva-

Tableau 1 Résultats du nombre de personnes vues pendant les prospections médicales actives, des séropositifs, et malades dépistés selon la localisation géographique et l'année.

Année	Localité	Nbre pers vues	Estimation du taux de présentation (%)	Nbre sé ropositifs	Séroprévalence (%)	Nbre de malades	Prévalence (%)
2000	Bonon	15 289	55	170	1,11	74	0,48
2001	Oumé	8071	40	46	0,57	7	0,09
2002	Bonon	8284	40	96	1,16	33	0,40
	Sinfra	3117	40	24	0,77	4	0,13
2003	Oumé	1011	30	16	1,58	1	0,1
	Doukouya, Guépahouo	2909	25	16	0,55	0	0
	Bonon	1369	27	25	1,83	2	0,15
	Bouaflé	1178	20	14	1,19	0	0
	Sinfra	7552	30	70	0,93	10	0,13
Total		48 747		477	0,98	131	0,27

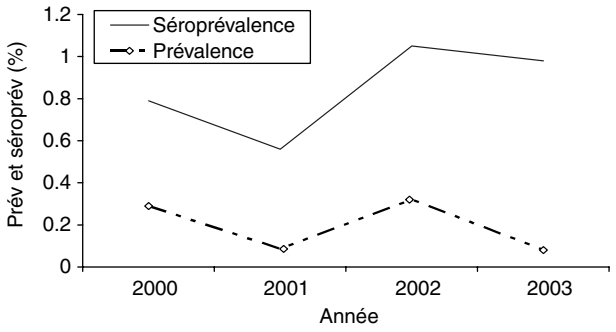

Figure 2 Evolution de la prévalence et de la séroprévalence au centre ouest de 2000 à 2003.

lence observés selon les foyers et l'année. Le foyer de Bonon est le plus touché (0,44% sur 24 942 personnes vues entre 2000 et 2003), suivi de celui de Sinfra et de Oumé. Mais ces résultats reflètent mal une réalité plus complexe: en affinant l'échelle d'analyse, par exemple sur le foyer de Bonon, d'importantes variations spatiales de prévalence entre le nord et le sud de la ville ont été mises en évidence (Solano *et al.* 2003), allant de 0% au nord à plus de 3% dans certaines localités du sud. De même à Oumé, 3 zones différentes ont été prospectées, dont 2 où aucun malade n'a été trouvé. La prévalence de la maladie dans la seule zone du village de Zangué, où ont été dépistés 7 malades en 2001 sur 1011 personnes vues, est donc plus précisément de 0,7%.

Aucun malade n'a été trouvé sur les sondages faits dans les régions de Bouaflé (ancien foyer historique), ainsi que de Doukouya, Guépahouo (situés entre Oumé et Sinfra) mais des séropositifs ont été trouvés. Parmi ces séropositifs du centre-ouest, 7 étaient des déplacés de guerre venus de Bouaké, Duekoué et Toulepleu. Il s'agit de personnes vivant dans des familles hôtes où existent d'autres séropositifs et même 2 des malades dépistés.

La Figure 2 illustre la variation de prévalence d'une année à l'autre, sur les sondages effectués, en regroupant les zones visitées. La prévalence varie généralement de 0,01 à 0,3%. Elle est similaire en 2000 et 2002, et en baisse apparente en 2001 et 2003. La baisse de 2001 est due au fait qu'une seule prospection ait eu lieu cette année là. En revanche, il apparaît plus difficile a priori d'expliquer la baisse apparente de 2003 alors que plusieurs prospections ont pu avoir lieu dans le centre-ouest. Il serait tentant de l'attribuer à une diminution réelle de la maladie suite aux prospections répétées dans ces régions. Mais d'autres facteurs peuvent jouer un rôle important, et nous allons tenter de les comprendre.

Taux de présentation des populations

Sur le Tableau 1, des variations de présence des populations aux prospections médicales sont visibles, avec notamment une diminution des taux de présentation pour l'année 2003. Nous avons cherché à voir s'il y avait des différences de taux de présentation des populations sur les centres dans lesquels nous sommes passés au moins 2 fois successivement. Par exemple, dans la ville de Bonon, un centre appelé « lisière Mady » a été visité en 2000, 2002, et 2003. On y constate une baisse progressive de la fréquentation globale (Tableau 2, $P < 0{,}0001$). Cette baisse est essentiellement due à une baisse du taux de présentation des populations Mossi de 2000 à 2003 ($P = 0{,}024374$).

Dans la région de Oumé, la variation de fréquentation est plus marquée entre 2001 et 2003 (Tableau 3, $P < 0{,}0001$), et la répartition par ethnie montre des taux remarquables: alors que les autochtones Gouro, mais aussi les Baoulé ont un taux de présentation globalement plus élevé, les Malinké, les Sénoufo et surtout les Mossi ne se sont pratiquement pas présentés.

En regroupant les résultats de quelques centres du foyer de Bonon avant septembre 2002 (soit les prospections de 2000 et de 2002), et après septembre 2002 (prospection de 2003) cette même tendance est confirmée: baisse significative de présentation des populations soudanaises aux

Tableau 2 Effectifs et proportions des populations vues au centre de « Lisière Mady » (Bonon) en 2000, 2002 et 2003

	2000		2002		2003		
Ethnie	Eff	% de vus	Eff	% de vus	Eff	% de vus	Test de Chi-carré
Mossi	356	42,33	265	32,22	184	43,44	$P = 0{,}024$
Malinké	249	29,61	213	31,87	182	34,92	N.S.
Sénoufo	88	10,46	70	10,51	60	11,48	N.S.
Baoulé	46	5,47	7	7,71	44	1,15	
Gouro	45	5,35	26	13,66	78	4,26	N.S.
Autres	57	6,75	25	4,03	23	4,10	N.S
Total	841	100,00	610	100,00	571	100,00	

N.S., non significatif.

Tableau 3 Effectifs et proportions des populations vues au centre de Zangué village (Oumé) en 2001 et 2003

	2001		2003	
Ethnie	Eff	%	Eff	%
Mossi	316	19,64	39	4,26
Malinké	41	2,55	8	0,87
Sénoufo	42	2,61	10	1,09
Baoulé	835	51,90	655	71,58
Gouro	213	13,24	149	16,28
Autres	162	10,07	54	5,90
Total	1609	100,00	915	100,00

$\chi^2 = 166{,}91$; $P = 0{,}000001$.

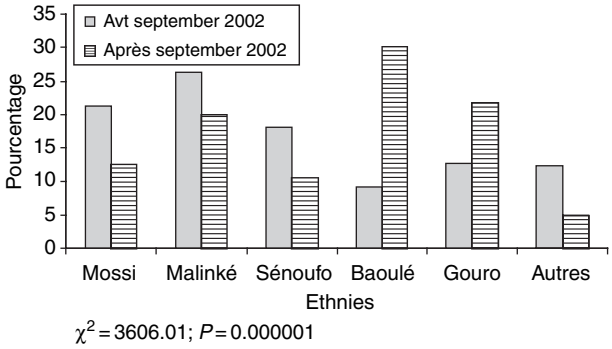

Figure 3 Evolution du pourcentage de personnes vues par ethnie avant et après le début des évenements sociopolitiques.

prospections post-septembre 2002 et augmentation significative de celle des Baoulé et Gouro (Figure 3).

De cette analyse se dégage un constat: c'est l'observation de deux blocs formés d'une part par les populations soudanaises et d'autre part par les Gouro et Baoulé, et dont les proportions de présence aux prospections médicales évoluent en sens inverse. Or, il faut rappeler que ces populations soudanaises, en particulier les Mossi du Burkina Faso constituent une population particulièrement touchée par la THA (Hervouët & Laveissière, 1987; Meda *et al.* 1993).

On observe par ailleurs que la participation des différentes ethnies varie selon l'ethnie du chef (de village, du quartier, d'une communauté donnée ou de famille) qui accueille l'équipe de prospection (Figure 4). Ainsi, dans l'ensemble des zones prospectées en 2003 après le début des événements, 90,5% (3045) des personnes présentes aux prospections 'organisées' par les Baoulé étaient des Baoulé, contre seulement 1,7% (56) de Mossi. Il en fut de même pour les prospections organisées en collaboration avec les Gouro ou les Gagou, ainsi qu'avec les Mossi. Ces derniers, en plus de leur faible présentation, ne se sont venus que lorsqu'ils avaient été impliqués dans l'organisation de la prospection. En revanche, on observe aussi que lorsque la prospection est conjointement organisée par plusieurs ethnies ou lorsque le centre de prospection est localisé sur un site neutre tel que le centre de santé ou l'école, toutes les ethnies se prèsentent dans des proportions équilibrées.

Dépistage passif

Entre 2000 et 2003, au total 250 cas ont été dépistés dont 131 (52.40%) au cours des prospections médicales actives et 119 (47.60%) de façon passive dans les centres. On note cependant qu'en 2003 le taux des cas dépistés passivement

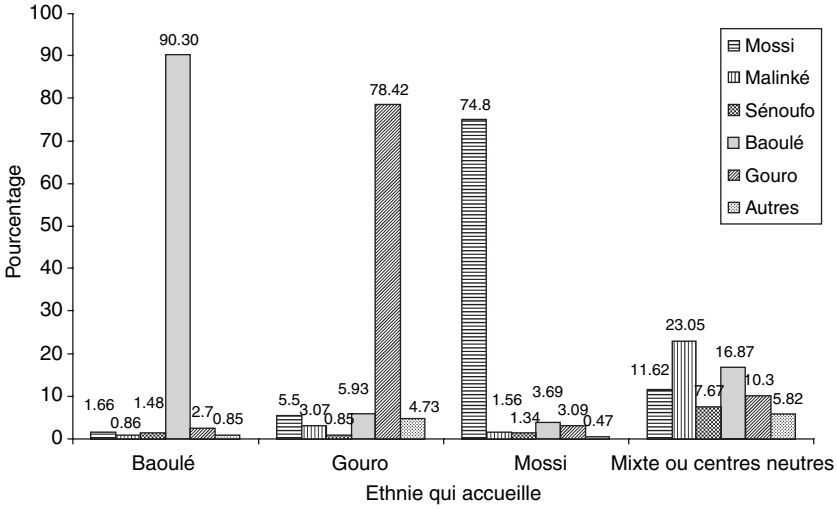

Figure 4 Proportion des ethnies vues aux prospections médicales en fonction de l'ethnie qui accueille l'équipe de prospection.

est significativement plus élevé qu'en 2002 malgré un sondage effectué dans tous les foyers actifs et étendu à de nouvelles zones (Guépaouo et Doukouya) et à des zones restées longtemps inexplorées (Bouaflé). Le regroupement des cas dépistés avant le début des événements sociopolitiques (en 2000, 2001 et 2002) et leur comparaison selon le mode de dépistage avec les cas dépistés en 2003, c'est à dire après le début des événements, montre, contrairement à ce qui est habituellement observé, qu'il y a eu significativement plus de cas dépistés passivement après le début des événements ($P = 0{,}000164$) (Tableau 4).

Discussion

La maladie du sommeil sévit toujours en Côte d'Ivoire, localisée essentiellement dans le centre-ouest avec des prévalences hétérogènes, variant d'un foyer à l'autre et d'une année à l'autre. Les résultats fournis dans ce travail ne reflètent pas la situation générale de la THA en Côte d'Ivoire, mais seulement celle de quelques foyers du centre-ouest irrégulièrement prospectés, et de manière non exhaustive d'une année à l'autre.

Dans ces foyers explorés, les taux de prévalence obtenus en 2003 sous–évaluent l'intensité de la maladie à cause du faible taux de présentation des populations à risque. En effet, cette étude montre que depuis les événements sociopolitiques en Côte d'Ivoire, les populations soudanaises, considérées comme étant les plus soumises au risque de cette maladie en Côte d'Ivoire (Meda *et al.* 1993), et habituellement plus présentes aux prospections médicales, ont significativement réduit leur présence. Malgré une participation plus importante des Baoulé et des Gouro, ethnies généralement moins touchées par la maladie que les Mossi, le taux de participation est resté globalement faible.

Il semble s'agir ici d'un impact réel négatif de la situation sociopolitique actuelle sur les sondages médicaux. En effet, depuis le début des événements sociopolitiques, la méfiance intercommunautaire, déjà existante souvent pour des raisons foncières (Chauveau & Bobo Koffi 2003), s'est accrue. Les différentes populations ne se sentent donc en sécurité qu'au sein de leur communauté ethnique surtout lorsque celle-ci est, à travers l'un des leurs (chefs, personne influente, agent de santé communautaire) impliquée dans l'organisation de la prospection médicale. La présence de ce dernier constitue ainsi un gage de sécurité pour toute la communauté. C'est ainsi que les participations, non seulement faibles, étaient sélectives liées à l'ethnie.

Tableau 4 L'ensemble des cas selon le mode de dépistage avant et après les événements sociopolitiques

	Passif		Actif		
Période	Effectif	%	Effectif	%	Total (%)
Avant événements	85	41,87	118	58,13	203 (100)
Après événements	34	72,34	13	27,66	47 (100)
Total	119	47,6	131	52,4	250 (100)

$\chi^2 = 14{,}20$; $P = 0{,}000164$.

Cette apparente baisse de prévalence de la THA, qui ne reflète en réalité qu'une baisse de présentation aux prospections médicales des populations les plus exposées, qui donc bénéficient d'une surveillance sanitaire encore moins importante qu'habituellement, pourrait constituer un partie de l'explication de l'idée classiquement admise, mais dont on ne connaît pas les mécanismes précis, selon laquelle l'épidèmisation de la THA est liée aux troubles socio-politiques (Prothero 1963; Moore *et al.* 1999).

Une autre conséquence liée directement à la guerre est constituée des déplacements massifs de population, voire des retours (temporaires ou définitifs) de certains allogènes vers leur pays d'origine. Cela pourrait également expliquer le faible taux de présence des soudanais. Même s'il est encore tôt pour avoir une idée de leurs conséquences en matière de THA, une vigilance accrue s'impose dans les zones d'accueil de ces réfugiés (Burkina Faso, par exemple) où l'équilibre endèmique pourrait s'en trouver bouleversé. Les quelques séropositifs trouvés ici qui étaient des sujets déplacés méritent par exemple d'être suivis.

En revanche, on note que, contrairement à ce qui est observé habituellement (en Côte d'Ivoire et en Guinée, Camara *et al.* 2005), les consultations passives en poste fixe ont permis de dépister significativement plus de cas en 2003 que les prospections médicales. Ces dépistages passifs ayant eu lieu après le passage des équipes mobiles, il s'agit possiblement d'une conséquence immédiate de la méfiance suscitée lors des prospections médicales. En effet, on peut penser que le surplus de personnes venues consulter dans les centres de traitement de la THA est constitué des personnes qui, se sentant malades, ont refusé de se présenter aux équipes mobiles pour les raisons évoquées plus haut. Ces personnes ont alors trouvé plus rassurant de consulter dans les centres de traitement de la THA.

Cela montre les limites des équipes mobiles pendant ces périodes de trouble: leur efficacité est moindre en terme de taux de présentation des populations les plus à risques et donc de nombre de cas dépistés. En revanche, dans ces périodes, la surveillance en poste fixe apparaît plus efficace et mériterait d'être renforcée.

Conclusion

Depuis le début des événements sociopolitiques de septembre 2002 en Côte d'Ivoire, l'évolution de la situation

de la THA devient difficilement apprèciable par les équipes mobiles. Le taux de présentation des populations à risque est non seulement significativement réduit mais aussi très sélectif lié à l'ethnie, reflétant probablement la situation conflictuelle intercommunautaire due au problème du foncier rural et à la situation générale de guerre que connaît le pays. Cette situation est marquée dans le centre-ouest, proche de l'ancienne ligne de front dénommée « zone de confiance », qui est une zone de forte immigration mais aussi un lieu de concentration des foyers les plus actifs de THA. Les prospections médicales en période de trouble, même lorsqu'elles sont possibles, restent donc peu efficaces dans ce contexte. Elles ont toutefois un impact positif indirect, suscitant apparemment un plus grand nombre de consultations en poste fixe.

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The impact of war on the evolution of sleeping sickness in west-central Côte d'Ivoire

OBJECTIVE To evaluate the situation of sleeping sickness in west-central Côte d'Ivoire from 2000 to 2003, in view of the war which broke out in September 2002.

METHODS Active surveys by medical teams and passive case detection.

RESULTS Between 2000 and 2003, 250 patients were diagnosed with sleeping sickness. At first it appeared that sleeping sickness prevalence had fallen since the beginning of political troubles. But this apparent drop was due to poor population coverage. Participation in medical surveys differed according to ethnic group, reflecting land use conflicts between ethnic communities. Such conflicts are common in this area, but have been exacerbated by the war.

CONCLUSION In war, assessing the importance of sleeping sickness by medical surveys only is very difficult. But detection of sleeping sickness cases by passive surveillance increased.

Keywords Human African trypanosomiasis, epidemiology, Côte d'Ivoire, war

El impacto de la guerra en la evolución de la enfermedad del sueño en el centro-oeste de Costa de Marfil

OBJETIVO Evaluar la situación de la enfermedad del sueño en el centro-oeste de Costa de Marfil entre 2000 y 2003, en vista de la guerra que comenzó en Septiembre del 2002.
MÉTODO Búsqueda activa con equipos médicos y detección pasiva de casos
RESULTADOS Entre el 2000 y el 2003 se diagnosticaron 250 pacientes con la enfermedad del sueño. En un principio parecía que la prevalencia de la enfermedad había caído desde el comienzo de la guerra. Pero esta caída aparente fue debida a una mala cobertura de la población. La participación en las encuestas médicas fue diferente dependiendo del grupo étnico, lo cual refleja el conflicto en el uso de la tierra entre comunidades étnicas. Estos conflictos son comunes en esta área, pero la guerra los ha exacerbado
CONCLUSIONES Valorar durante la guerra la importancia de la enfermedad del sueño solamente mediante encuestas médicas es muy difícil. La detección de la enfermedad del sueño por detección pasiva de casos, sin embargo, ha aumentado.

Palabras clave tripanosomiasis humana africana, epidemiología, costa de marfil, guerra

Genetic and Morphometric Evidence for Population Isolation of *Glossina palpalis gambiensis* (Diptera: Glossinidae) on the Loos Islands, Guinea

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ABSTRACT Allele frequencies at four microsatellite loci, and morphometric features based on 11 wing landmarks, were compared among three populations of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in Guinea. One population originated from the Loos islands separated from the capital Conakry by 5 km of sea, and the two others originated from the continental mangrove area close to Dubreka, these two groups being separated by ≈30 km. Microsatellites and wing geometry data both converged to the idea of a separation of the Loos island population from those of the mangrove area. Although occasional contacts cannot be excluded, our results support the hypothesis of the Loos population of tsetse flies being a completely isolated population. This situation will favor a sequenced intervention against human African trypanosomosis and the possibility of an elimination of tsetse from this island.

KEY WORDS *Glossina palpalis*, microsatellite DNA, geometric morphometrics, wings, Guinea

Human African trypanosomosis (HAT, or sleeping sickness) is showing some signs of declining due to recent efforts on case detection and treatment, notably in Central Africa (Jannin 2005). However the situation in West Africa is much less clear, and Guinea and Côte d'Ivoire are thought to be the two countries most affected by this disease (Camara et al. 2005, Kaba et al. 2006). Guinea has a long history of sleeping sickness, which was particularly prevalent in the years 1930–1940 (Brengues et al. 1964). Current data show prevalences up to 2–5% in villages of the coastal mangrove area (Dubreka focus) (Camara et al. 2005). The Loos islands are completely separated from this mangrove area of the mainland. The situation of HAT in these islands is not currently known, but there are historical reports of the disease: in 1942, a medical survey conducted by Med. Cap. Héricord detected 30 patients. In 1944, out of 1,924 inhabitants who were registered from the islands, 1,627 were visited, and 16 cases were detected (15 on Kassa island and one on Fotoba island). After that, little information has been available except for seven cases originating from these islands between 1971 and 1987; these cases were passively detected and treated in Dubreka. It is not known whether the disease was autochtonous or was imported from other localities.

In West Africa, HAT is mainly transmitted by the tsetse fly species *Glossina palpalis* Van der Planck (Diptera: Glossinidae). Control of tsetse can be achieved through a variety of techniques, including traps, insecticide impregnated targets, live-baits, sequential aerial spraying, and sterile male release (Cuisance et al. 1980). Generally, however, the tsetse populations then tend to recover, due to either flies surviving the initial interventions, migrant flies coming 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 neighboring localities is of low likelihood. This end is most readily achieved for isolated island populations, as shown by the elimination of *Glossina pallidipes* Austin from the Island of Principe in 1914 (Da Costa et al. 1916), and the elimination of *Glossina austeni* Newstead from Unguja Island of Zanzibar in 1997 (Vreysen et al. 2000). 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 subpopulations. In essence, the

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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 (Patterson and Schofield 2005). Initial studies already showed evidence of strong structuring of *G. palpalis* populations in fragmented landscapes (Solano et al. 2000). With more detailed study, it should therefore be possible to determine key areas where tsetse control interventions can proceed with relatively low risk of reinvasion from neighboring areas.

To examine the population structure of *G. palpalis*, we used two approaches in the current study, one approach based on genetic variation at microsatellite DNA loci and the other approach based on phenetic variation as described by the geometry of the wings. The main objective was to assess whether the tsetse population from Loos islands was isolated from two other populations of the mangrove area of the HAT focus of Dubreka.

Materials and Methods

Study Area. The HAT focus of Dubreka is located ≈45 km from Conakry, the capital of Guinea. HAT has shown incidences up to 5% in some of the visited villages from 1997 to 2005. The area is situated among the coastal mangrove, with anthropic Guinean savannah, and permanent or temporary inundated areas. Near the town of Dubreka (25,000 inhabitants), people live in villages of between 300 and 2,000 inhabitants, fragmented in many smaller localities. Main activities include fishing, salt extraction, and agriculture ("vergers" of *Elaeis guineensis*, mangoes, rice, and food crops). Loos islands are separated from the mainland, by 5 km of sea at the shortest distance. But the first mangrove area where tsetse occur are at ≈20 km from these islands (Fig. 1).

Entomological Surveys. In May 2005, six Vavoua traps (Laveissière and Grébaut 1990) were placed on Fotoba island (Loos islands), and 23 traps were placed in two mainland localities of the littoral (Magnokhoun) and mangrove (Touguissoury) areas. These two mainland localities are separated by ≈15 km, and the shortest distance between them and Loos islands is 30 km. Cages were changed daily during 2 to 4 days, and tsetse were counted and separated by sex. From each dissected tsetse, the wings were removed and put in individual, labeled, dry Eppendorf tubes, and three legs were removed and put in individual, labeled, dry Eppendorf tubes.

Microsatellite Loci. In total, 71 individuals were used for the genetic analyses at microsatellite loci: 23 in Loos islands (14 males [M], nine females [F]), 28 in Magnokhoun (14 M, 14 F), and 21 in Touguissoury (10 M, 10 F).

Four microsatellite loci were analyzed: Gpg55,3 (Solano et al. 1997); pgp11and pgp1 (Luna et al. 2001), and B104 (kindly provided by A. S. Robinson, IAEA, Vienna, Austria). Locus Gpg55,3 has been reported to be located on the X chromosome (Solano et al. 1997, Gooding et al. 2004), and given an absence of heterozygotes on a subsample of males (data not shown), B104 and pgp11 also were interpreted to be located on the X chromosome.

To each tube containing the legs of the tsetse, 200 μl of 5% Chelex chelating resin was added (Walsh et al. 1991, Solano et al. 2000). After incubation at 56°C for 1 h, DNA was denatured at 95°C for 30 min. The tubes were then centrifuged at 12,000 × *g* for 2 min and frozen for later analysis.

The polymerase chain reaction (PCR) reactions were carried out in a thermocycler (MJ Research, Cambridge, United Kingdom) in 10-μl final volume, by using 1 μl of the supernatant from the extraction step. After PCR amplification, allele bands were routinely resolved on a 4,300 DNA Analysis System from LI-COR (Lincoln, NE) after migration in 96-lane reloadable (3×) 6.5% denaturing polyacrylamide gels. This method allows a multiplex by the use of two infrared dyes (IRDye), separated by 100 nm (700 and 800 nm), and read by a two-channel detection system that uses two separate lasers and detectors to eliminate errors due to fluorescence overlap. To determine the different allele sizes, a large panel of ≈30 size markers was used. These size markers had been previously generated by cloning alleles from individual tsetse flies into pGEM-T Easy Vector (Promega, Madison, WI). Three clones of each allele were sequenced using the T7 primer and the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Sequences were analyzed on an Applied Biosystems 310 automatic DNA sequencer, and the exact size of each cloned allele was determined. PCR products from these cloned alleles were run in the same acrylamide gel as the samples, allowing the allele size of the samples to be determined accurately.

Microsatellite Data Analysis. For the total sample subdivided into the three localities, Wright's F_{is} (within sample heterozygote deficiency, a measure of deviation from panmixia) and F_{st} (measure of population differentiation) were estimated using Weir and Cockerham's unbiased estimators (f for F_{is}, θ for F_{st}) (Weir and Cockerham 1984). For random mating (within samples) or random distribution of individuals (between samples), F values are expected to be zero. When F_{st} was measured, it was compared with $F_{st\ max} = 1 - H_s$ (Hedrick 1999, 2005).

The significance of F_{is} (deviation from panmixia) at each locus, and over all loci, also was tested separately within each sample by using 10,000 permutations of alleles between individuals. Males were hemizygous at loci on the X chromosome. For these loci, measure of F_{is} and its significance were conducted only on females. The significance of F_{st} (population differentiation) was assessed using 10,000 permutations of genotypes among samples. To evaluate significance when multiple tests were performed, the sequential Bonferroni procedure was applied (Rice 1989).

An unweighted pair-group method with arithmetic average (unweighted pair group method with arithmetic mean) dendrogram was built based on Cavalli-Sforza and Edwards (1967) chord distance between the three populations. This distance is indeed the most

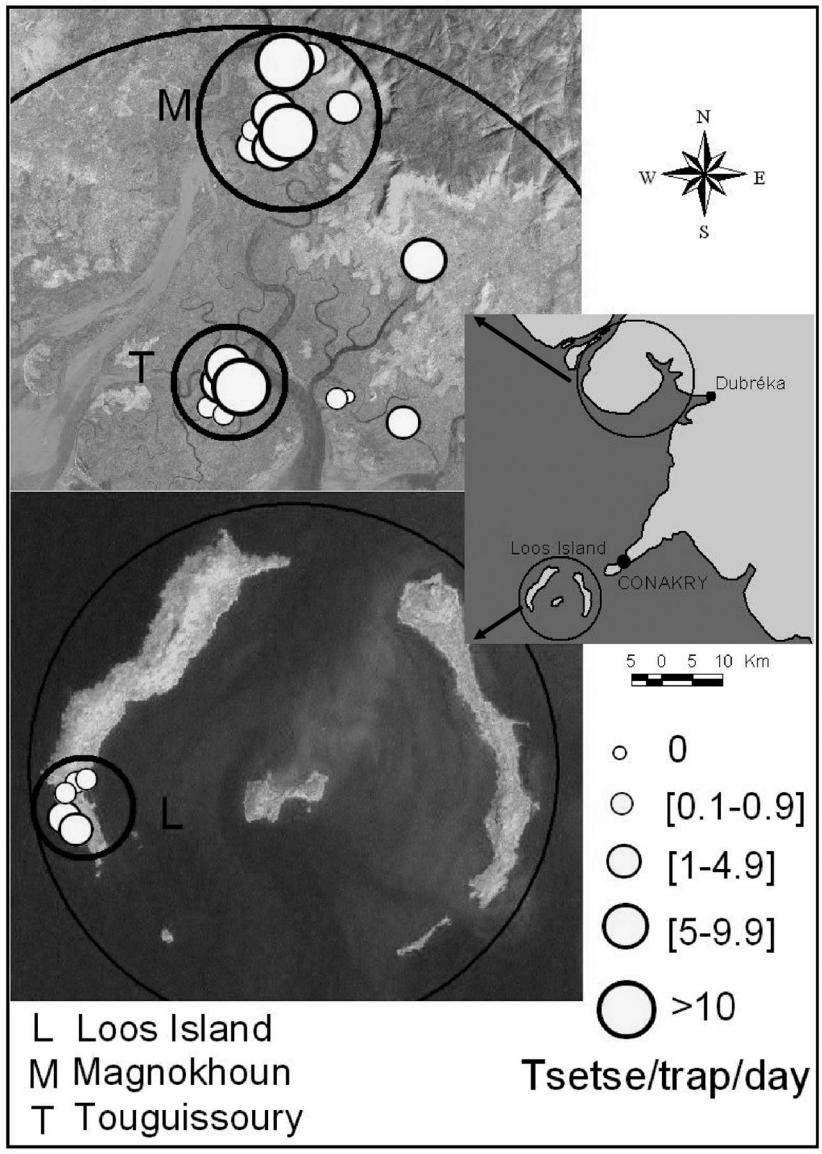

Fig. 1. Geographic location of study area. The circles correspond to the three localities of the tsetse samples. L, Loos islands; M, Magnokhoun; and T, Touguissoury.

appropriate for tree construction (Takezaki and Nei 1996).

Morphometrics. Out of the 71 individuals submitted to microsatellite analyses, 64 showed wings in good state for morphometric studies. Wings were dry-mounted between two microscope slides and scanned at 3,200 dpi. On this image, 11 landmarks defined by vein intersections were recorded (Fig. 2). Their coordinates were subjected to generalized Procrustes analysis (GPA) (Rohlf 1990, 1996). Centroid size (Bookstein 1991) was used to describe size changes among sexes and localities (Fig. 3).

For geographic comparisons, 18 "partial warps" (PW), corresponding to 11 landmarks (Fig. 2) were computed from the right wings by using the total sample, mixing males and females: 18 individuals from Loos islands (10 M, 8 F), 24 from Magnokhoun (13 M, 11 F), and 22 from Touguissoury (13 M, 9 F). To circumvent the problem of small sample sizes relative to the large number of variables (18 PW), the 11 first "relative warps" (principal components of the PW) were used instead, representing >95% of the total shape variation. The residual allometry was estimated by multivariate regression of PW on size, on the total sample and separately in each sex, and statistical significance estimated by 1,000-runs permutation tests (Good 2000). To estimate the contribution of size variation to the geographic distinction provided by the discriminant functions, each of these was regressed on size variation (Fig. 4). The Mahalanobis distances were

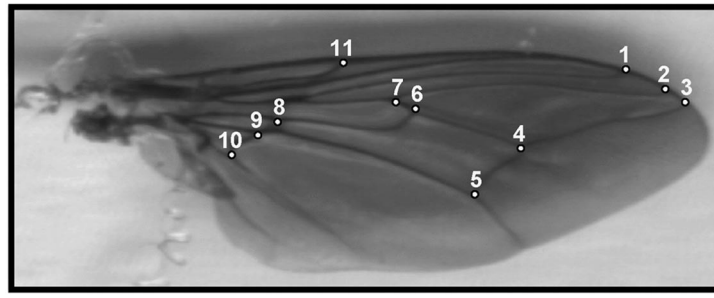

Fig. 2. Location of the 11 landmarks that were recorded for each tsetse wing.

examined for significance by permutation tests (1,000 runs) and used to construct an unweighted pair-group method with arithmetic average dendrogram. Based on these distances, the percentage of correctly assigned individuals was also computed for each locality.

For bilateral differences, only a subset of the total sample was used (9 M, 6 F from Loos, 8 M and 5 F from Magnokhoun, and 11 M and 7 F from Touguissoury). Five landmarks could be retained (Fig. 2, see landmarks 1, 3, 5, 10, and 11). To provide a better estimate of digitizing error, both wings of each individual were recorded three times (Møller and Swaddle 1997).

Size asymmetry was estimated on the basis of centroid size and followed the analysis of variance (ANOVA) procedure recommended by Palmer and Strobeck (1986). In the absence of significant directional asymmetry, the distribution of signed differences was examined for kurtosis to assess the existence of fluctuating asymmetry (or reject the existence of antisymmetry).

Software. The F_{is} and F_{st} estimators were calculated with FSTAT version 2.9.3 software (Goudet 1995). Cavalli-Sforza and Edwards (1967) chord distances were computed by the GENETIX version 4 software package (Laboratoire Génome et Populations, Centre National de la Recherche Scientifique Unité Propre de Recherche 9060, Université de Montpellier II, Montpellier, France).

Collection of anatomical landmarks, GPA, multivariate analyses as well as asymmetry detection and measurement were performed using software freely available at http://www.mpl.ird.fr/morphometrics (developed by J.P.D.).

PHYLIP package (by J. Felsenstein, http://evolution.genetics.washington.edu/phylip.html) was used to construct the unweighted pair-group method with arithmetic average tree, and NJPLOT (http://pbil.univ-lyon1.fr) was used for tree edition (Perrière and Gouy 1996).

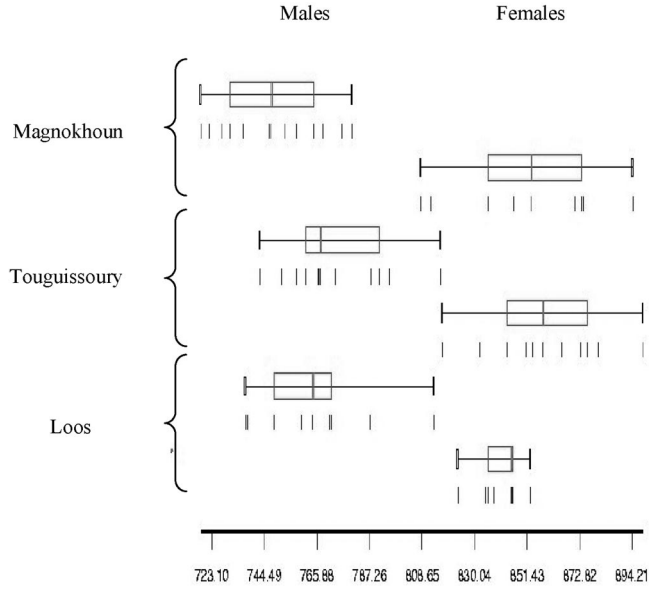

Fig. 3. Quantile plots showing in each sex the distribution of individuals along the isometric estimator of size (centroid size). Each box shows the group median separating the 25th and 75th quartiles, with the 10th and 90th quartiles shown as lines on the right and left sides of the box.

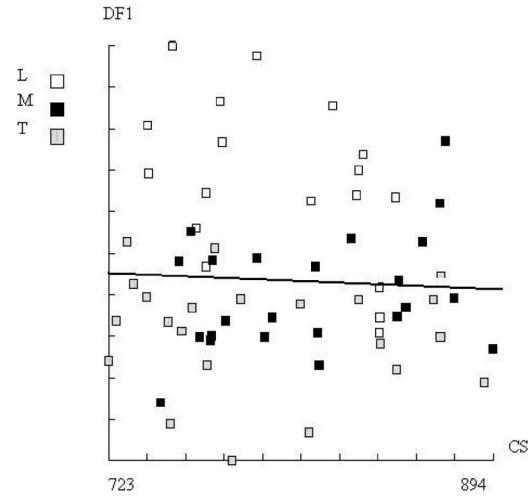

Fig. 4. Regression of first discriminant factor on centroid size. Vertical axis is the DF1, representing 84% of the total discrimination. Horizontal axis is the centroid size of the wing, in pixels. White squares, Loos (L); black squares, Magnokhoun (M); and gray squares, Touguissoury (T). Regression line is shown.

Results

Entomological Surveys. Of 421 tsetse (240 M, 181 F) that were trapped during the survey, all were *G. p. gambiensis* based on morphological characters and on geographical distribution. The apparent density (AD) averaged 4.53 tsetse per trap per day. On Loos islands, 26 tsetse in total were caught (AD of 1.44 tsetse per trap per d), 277 were caught at Magnokhoun (AD of 6.15 tsetse per trap per d), and 118 at Touguissoury (AD of 4.07 tsetse per trap per d).

Genetics. From the 71 individuals analyzed, the number of alleles at each locus was 18, 13, 16, and 14 for loci B104, Pgp11, Pgp1, and Gpg55,3 respectively (Table 1). The Loos sample had the lowest number of alleles. Weir and Cockerham (1984) estimators indicated an overall F_{is} of 0.15 ($P < 0.001$) and an averaged F_{is} per population of 0.18, 0.16, and 0.13, respectively, for Loos, Magnokhoun, and Touguissory, none of these values being significant (Table 2).

Overall F_{st} averaged +0.032 ($P < 0.0001$), indicating genetic differentiation between the three populations. When measured among the three populations taken by pairs, pairwise F_{st} were 0.014 between Magnokhoun and Touguissory, 0.032 between Loos and Magnokhoun, and 0.062 between Loos and Touguissory.

Because F_{st} between Magnokhoun and Touguissory was low and nonsignificant, these two samples were mixed into one population and compared with Loos. The resulting F_{st} was 0.057 (highly significant, $P < 0.0001$).

Because H_s was 0.832, $F_{st\ max} = 1 - H_s$ was 0.168, and our maximum F_{st} value was 0.057. A standardized estimate of F_{st} would thus give $F_{st'} = F_{st}/F_{st\ max} = 0.34$. Should this value have been close to 1, a complete lack of migrant would have been supported. The lower value (0.34) suggests that either there are migrants, or there have been migrants in the past and the separated populations did not yet reach equilibrium. If equilibrium is assumed in a two island model, then the corresponding number of migrants would be $Nm = (1 - F_{ST})/8F_{ST}$, which would give, with our "standardized" measure of F_{ST}, $Nm = 0.24$ migrant per generation or one migrant every four generations.

Morphometrics. A strong sexual dimorphism for size was disclosed in each locality, in favor of females (Fig. 3). Size variation was not significant between localities.

The multivariate regression of PW on size was significant on the total sample, mixing males and females (1,000 runs, $P = 0.0040$), but it was not significant within each sex (1,000 runs, $P = 0.508$ in males and $P = 0.083$ in females). This suggests that allometric effects were mainly due do sexual size dimorphism.

The first discriminant function (DF1, 84% of the total variation) clearly separated the Loos population from the other two populations (Fig. 4), whereas these latter populations were slightly separated by the second discriminant function (DF2, 16% of the total variation). Comparing flies from Loos islands with the group formed by flies from Magnokhoun and Touguissory, the reclassification scores were 77% for Loos and 91% for mainland group. Size contribution to the geographic separation provided by DF1 was not significant (Fig. 4; $r^2 = 0.001$, $P > 0.05$).

Bilateral differences of centroid size showed distinct patterns in males and females, varying with geography. In females, directional and nondirectional asymmetries were found to be significant for the Loos population only. This suggests that nondirectional asymmetry on the island was antisymmetry, although kurtosis could not be detected. In females of the continental area as well as in males from the three localities, nondirectional (but no directional) asymmetry was detected, at a slightly higher amount in males (Table 3), and no kurtosis was detected in the distri-

Table 1. Gene diversity and number of alleles sampled by locus by population

Locus	Gene diversity/locus/pop			No. alleles sampled		
	L	M	T	L	M	T
B104	0.917	0.937	0.939	8	12	12
Pgp11	0.764	0.846	0.811	6	9	7
Pgp1	0.918	0.926	0.833	10	12	7
Gpg55,3	0.830	0.824	0.800	5	11	10

L, Loos islands; M, Magnokhoun; and T, Touguissoury.

Table 2. F_{is} per locus per population

Locus	F_{is}/locus/pop			P value (0.05 level)		
	L	M	T	L	M	T
B104	0.377	0.161	−0.065	0.02	0.05	1.00
Pgp11	0.127	0.071	0.137	0.40	0.39	0.32
Pgp1	0.109	0.064	0.333	0.20	0.36	0.04
Gpg55,3	0.097	0.353	0.125	0.42	0.0042	0.28
Total	0.182	0.159	0.126	0.017	0.013	0.013

Table 3. Left-right comparisons between the three tsetse populations

	Directional asymmetry	Nondirectional asymmetry
Females		
Loos	34**	20**
Magnokhoun	ns	8*
Touguissoury	ns	8*
Males		
Loos	ns	16**
Magnokhoun	ns	16**
Touguissoury	ns	29**

Values are mean squares (MS) of an ANOVA output with individuals, side and their interaction as effects, and centroid size as dependent variable. ns, not significant (value not shown).**, $P < 0.00001$; *, $P < 0.0010$.

bution of signed differences. These results were compatible with fluctuating asymmetry in males and in females, although the nondirectional asymmetry in the island females suggest antisymmetry as the cause of their nondirectional asymmetry.

Classification Trees. The Mahalanobis distances (Dm) derived from shape variation of the wings of the three populations were significant only when comparing the Loos population with Magnokhoun (Dm = 1.82, $P = 0.014$) or Touguissory (Dm = 2.30, $P < 0.001$), so that the resulting unweighted pair-group method with arithmetic average tree produced a pattern isolating the tsetse from the island and grouping the tsetse from the mangrove localities (Dm = 1.09, $P = 0.450$) (Fig. 5, left). The unweighted pair-group method with arithmetic average tree based on the Cavalli-Sforza and Edwards chord distances (D_{cve}) at microsatellite loci between the three populations gave similar branching (Fig. 5, right), and significant values were again found only when comparing the Loos population with populations of the mainland (1,000 permutations, $D_{cve} = 0.13$, $P = 0.001$ between Loos and Magnokhoun; $D_{cve} = 0.140$, $P = 0.002$ between Loos and Touguissory; and $D_{cve} = 0.085$, $P = 0.141$ between Magnokhoun and Touguissory).

Discussion

This study was undertaken to explore the population structure of *G. p. gambiensis*, the main tsetse species found in the very active HAT focus of Dubreka, Republic of Guinea. We feel that knowing the genetic structure of a vector population is useful for understanding an epidemic and will contribute to a rational control operation. Adapted tools are genetic markers like microsatellite DNA markers (Jarne and Lagoda 1996), which have proven successful for population studies of many arthropod species, including insect vectors (Lanzaro et al. 1995, de Meeüs et al. 2002) and tsetse (Solano et al. 1999, Gooding and Krafsur 2005). In the current study, the use of morphometrics was explored as a complementary, low-cost tool, to get information on population structure (Dujardin and Slice 2006). Thus, allele frequencies at four microsatellite loci, and morphometric features based on 11 wing landmarks, were compared among three populations of *G. p. gambiensis*, one originating from an island located 5 km from the capital Conakry, and the two others from the continent in the HAT focus of Dubreka, these two groups being separated by ≈30 km.

The number of traps (six in Loos islands, a total of 23 in the two other localities) and the time during which they were left (between 2 and 4 d) do not allow comparison of tsetse densities between localities. It may explain the limited number of individuals of sample L available for genetic and morphometric comparisons. Adapted statistics were used based on nonparametric tests.

Overall, and within each of the three populations, F_{is} values were positive, indicating within population heterozygote deficiency. We attributed this apparent heterozygote deficiency mainly to the occurrence of null alleles, as suspected by the high variance of F_{is} values among loci for each population, and as it has previously been reported in tsetse (S.R., unpublished data). Thus, we assume that there was random mating within each population.

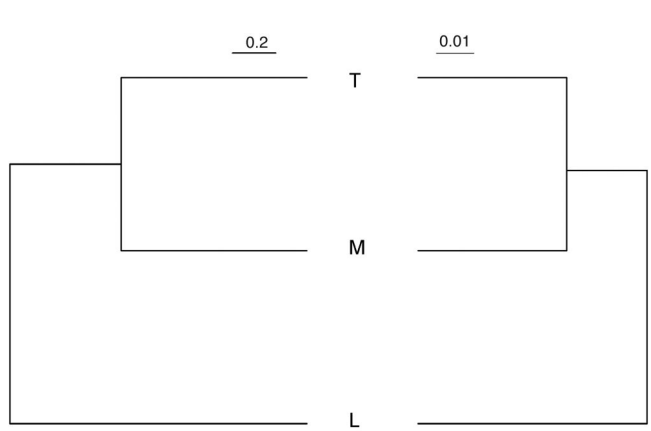

Fig. 5. Unweighted pair-group method with arithmetic average tree on genetic distances based on wing morphometry (on the left, Mahalanobis distance) and on microsatellite DNA loci (on the right, Cavalli-Sforza and Edwards distance) of the three tsetse populations.

The F_{st} value measured among the three populations was positive and significant, indicating genetic differentiation among the three populations. Pairwise Cavalli-Sforza and Edwards chord distances always distinguished the sample from Loos islands from the two other samples, whereas this same distance was the lowest and was not significant when measured between the two samples from the mangrove. When the two mainland populations were grouped and compared with the those from the island, F_{st} value was nearly 2 times higher (0.057) than the value comparing the three populations (0.032). Taking into account the high degree of polymorphism of our microsatellite loci, and using the suggestion of Hedrick (1999, 2005) for a standardized estimate ($F_{st'} = F_{st}/F_{st\ max}$ with $F_{st\ max} = F_{st}/(1 - H_s)$ provided a corrected estimate of 0.34. Despite being relatively high, this upper bound seems far from 1, suggesting that either there are some migrants (e.g., one effective migrant each four generations), or there have been migrants in the past, and the separated populations did not yet reach equilibrium. According to mark–recapture experiments on *G. palpalis* (Cuisance et al. 1985, Bouyer et al. 2007), flies should be able to reach a distance of 30 km if the populations had been separated by homogeneous riverine forest. But given the geographic location of our study, it seems unlikely for them to actively disperse from the islands to the mangrove or vice versa. However, passive transport by the numerous boats in the area cannot be ruled out. Our working hypothesis is that tsetse from the mangrove colonized the Loos islands probably by passive transport, and probably at the time when the islands harbored important economic activities such as the bauxite exploitation, and the Conakry peninsula harbored natural mangrove vegetation. The frequency of passive tsetse exchanges probably dropped with the regression of economic activities and the degradation of this vegetation. According to that hypothesis, both low migration rates and small population sizes would have contributed to the observed genetic differentiation.

Metric properties are under the influence of both environmental and genetic factors, and as continuous traits they are among the earliest characters to change between physically separated populations (Falconer 1981). Environment typically acts primarily on size (Glasgow 1961) and then on shape, frequently as an allometric effect of size change (Dujardin and Le Pont 2004). The three populations were not distinct on the base of size, but they could be discriminated by shape variation. In our data, although some residual allometry was found due to sexual dimorphism typical of tsetse species (Sané et al. 2000, de La Rocque et al. 2002), no allometric effect was detected to explain the shape discrimination between island and mainland populations. Pure shape differences among conspecific populations are more likely to result from adaptive or genetic causes than from environmental effects (Dujardin and Slice 2006). This interpretation of metric variation was supported by the detection of directional asymmetry (and possibly antisymmetry) only in the island population, although limited to the females. Directional asymmetry is a heritable trait very common in Diptera (Klingenberg et al. 1998), and the finding of intraspecific variability in this trait also suggests genetic differences.

Data from microsatellites and from wing geometry both converged to the idea of a separation of the Loos island population from the mainland. The level of separation in terms of number of migrants per generation seems high, which conforms to the known dispersing behavior of the insect. Although occasional contacts cannot be excluded, our working hypothesis is that the Loos population of tsetse flies is a completely isolated population. If true, this situation will favor control interventions, with the possibility of eliminating tsetse from this island.

Acknowledgments

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The tsetse fly *Glossina palpalis palpalis* is composed of several genetically differentiated small populations in the sleeping sickness focus of Bonon, Côte d'Ivoire

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Abstract

Glossina palpalis is the main vector of human African trypanosomosis (HAT, or sleeping sickness) that dramatically affects human health in sub-Saharan Africa. Because of the implications of genetic structuring of vector populations for the design and efficacy of control campaigns, *G. palpalis palpalis* in the most active focus of sleeping sickness in Côte d'Ivoire was studied to determine whether this taxon is genetically structured. High and statistically significant levels of within population heterozygote deficiencies were found at each of the five microsatellite loci in two temporally separated samples. Neither null alleles, short allele dominance, nor trap locations could fully explain these deviations from random mating, but a clustering within each of the two samples into different genetic sub-populations (Wahlund effect) was strongly suggested. These different genetic groups, which could display differences in infection rates and trypanosome identity, were composed of small numbers of individuals that were captured together, leading to the observed Wahlund effect. Implications of this population structure on tsetse control are discussed.

Keywords: *Glossina palpalis*; Sleeping sickness; Côte d'Ivoire; Wahlund effect; *T. brucei gambiense*; Microsatellites; Population structure

1. Introduction

Tsetse flies (Diptera: Glossinidae) are the main vectors of trypanosomes (Kinetoplastida: Trypanosomatidae), which cause human and animal trypanosomoses in tropical Africa. These diseases, after years of neglect, are strongly re-emerging and currently have a considerable impact on public health and economic development in sub-Saharan Africa (WHO, 2001; Louis, 2001), although there are finally recent signs of declining following a considerable intervention effort based on case treatment and surveillance (Jannin, 2005).

As with all vector-borne diseases, trypanosomosis transmission is complex and requires at least three interacting organisms: the vertebrate host, the insect vector, and the pathogen. Interruption at any point in these interactions can potentially reduce disease transmission. Unfortunately, it has not been possible to develop vaccines against trypanosomes, because African trypanosomes frequently change the antigenic nature of their surface proteins. Current control efforts rely primarily on active surveillance and treatment. These efforts, however, are hampered by population mobility, poor sensitivity of diagnostic tests, and lack of low-cost, efficacious drugs that have minimal adverse side effects.

Since trypanosome transmission relies on tsetse flies, eliminating this vector appears to be the most effective strategy to break the disease cycle. This presupposes a thorough knowledge of the epidemiology of the disease, including the

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biology, ecology, and population genetics of the targeted tsetse species and subspecies. However, our knowledge of tsetse still suffers from many gaps. For instance, it is widely acknowledged that genetic variation among vector populations probably affects the transmission of many parasitic diseases at a macrogeographic level (Lanzaro and Warburg, 1995; Gooding, 1996; Awono-Ambene et al., 2004), but little information is available to assess the spatial scale of this phenomenon. Tsetse flies have been reported to have a short flight duration (totalling 15–30 min/day), and may move, on average, from 200 m to 1.4 km/day (Leak, 1999), this mobility being very dependent on microhabitat conditions, in particular humidity. However, compared to this high daily mobility, Cuisance et al. (1985) reported that most *Glossina palpalis gambiensis* have a net lifetime displacement of no more than 1or 2 km (in a humid savannah area); the exceptions are some old females that can disperse more than 5 km. Data are scarce for forest species, but Gouteux et al. (1983) reported lifetime dispersal of a few kilometres for *Glossina palpalis palpalis*.

Glossina palpalis sensu lato is the most important vector of human African trypanosomosis (HAT or sleeping sickness) in West Africa, and is also one of the most important vectors of animal trypanosomoses. Two subspecies of *G. palpalis* are generally recognized: *G. p. gambiensis* and *G. p. palpalis*. The former lives in humid savannah, and the latter in forested areas; their distribution limits follow more or less the savannah–forest transition (Challier et al., 1983; see also Fig. 1). Preliminary work on *G. p. gambiensis* in Burkina Faso using microsatellite markers detected a genetic structuring of populations that may have an impact on the ability of this subspecies to transmit

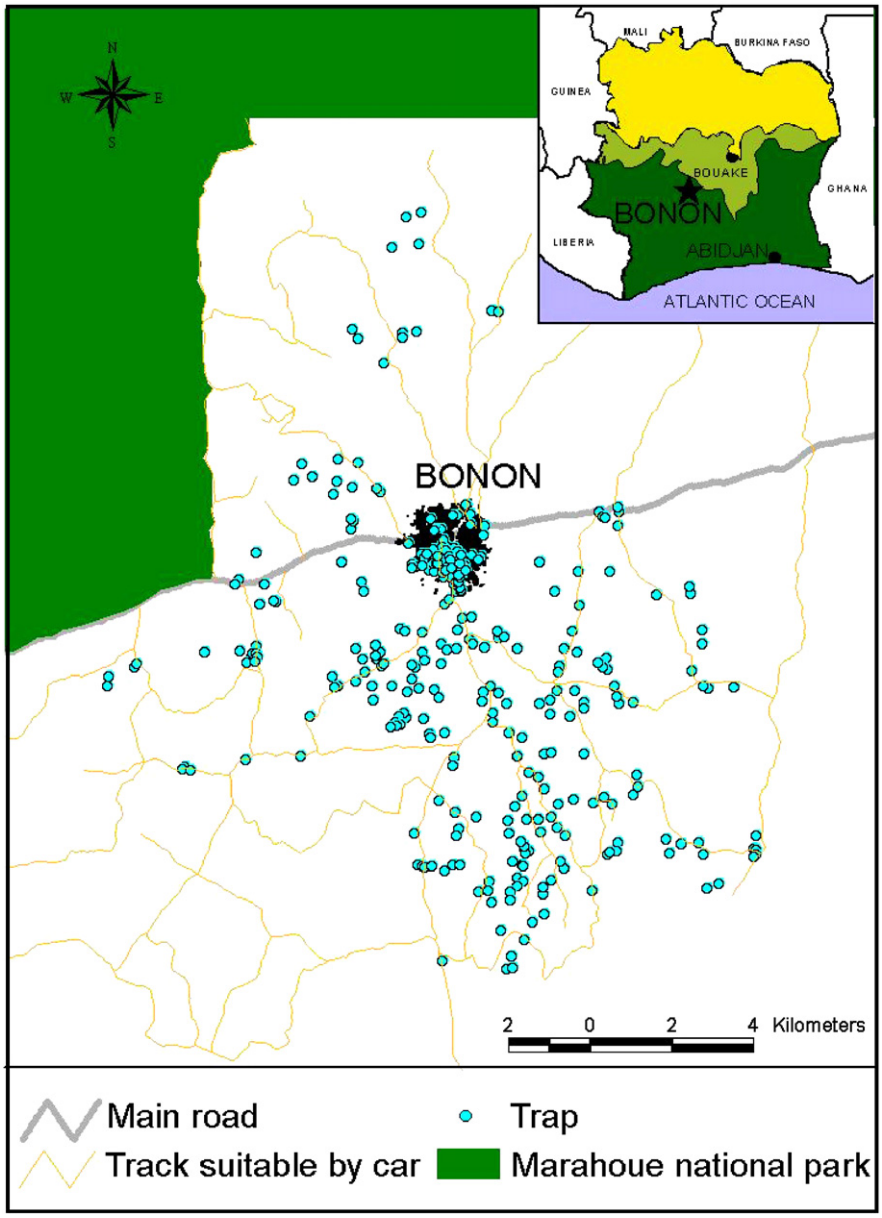

Fig. 1. Geographic location of the study area and the traps. The sleeping sickness focus of Bonon is located in the Center West of Côte d'Ivoire, just under the savannah–forest transition zone, and a few kilometres south of the reported limit of the two subspecies of *Glossina palpalis* (Challier et al., 1983).

animal trypanosomes (Solano et al., 2000). The objective of the present work was to investigate population genetics of *G. p. palpalis*, the main vector of HAT in West African forest zones, and its possible consequences for the epidemiology and control of this disease in an active sleeping sickness focus in west-central Côte d'Ivoire.

2. Materials and methods

2.1. Study area

The HAT focus of Bonon is located in west-central Côte d'Ivoire (6°55′N–6°W). Although the area is located in the forest area, near the forest–savannah transition (see Fig. 1), the forest has nearly been replaced by cocoa/coffee plantations and other crops. This new agro-ecosystem favours *G. palpalis* over other tsetse species because of the broad host preferences of *G. palpalis* (Gouteux et al., 1982; Reid et al., 2000). A few kilometres to the north the savannah area begins, and there *G. p. gambiensis* is found (Challier et al., 1983).

Beginning in April 2000, an HAT survey was conducted (Solano et al., 2003). Between 2000 and 2003, 130 cases of HAT were detected in this focus, giving an infection rate of about 0.45% (Kaba et al., 2006), which makes the area of Bonon the most active HAT focus of the country. After the first medical survey of 2000, two entomological surveys were conducted in November 2000 and January 2001. These tsetse samples will be referred to in the text as sample 1 and sample 2 for November 2000 and January 2001, respectively.

2.2. Entomological survey, tsetse dissection, sample collection

Following the first medical survey which detected 74 patients (Solano et al., 2003), the key epidemiologically significant sites (home, water supply points, and working places) relevant to each patient were recorded by Global Positioning System (GPS). Subsequently 320 Vavoua traps (Laveissière and Grébaut, 1990) were placed in those areas frequented by the patients, which represent a total surface area of about 15 km × 15 km. Each trap was maintained for four days, with cages changed daily, and the number of tsetse and sex ratio recorded.

At the field camp, after removing the tsetse from the trap, individual *G. p. palpalis* were processed as follows:

Three legs were removed and stored in dry Eppendorf tubes for subsequent genetic analyses.
Age of females was determined by dissection of the ovaries (Challier, 1965).
The mouthparts, salivary glands, and midgut were dissected to detect trypanosome infections by microscopy.
When a tsetse was found infected with trypanosomes, each of the three organs was collected separately in Eppendorf tubes containing 50 μl sterile distilled water. This material was used for PCR identification of trypanosomes using taxon-specific DNA primers (Masiga et al., 1992; Majiwa et al., 1994; Solano et al., 1995), including a microsatellite marker specific for *T. b. gambiense* group 1 (Truc et al., 2002). The details of techniques and results regarding identification of trypanosomes by PCR in the tsetse have been reported in Jamonneau et al. (2004).

2.3. PCR reactions at microsatellite loci

DNA was extracted from 122 females and 2 males from sample 1 (originating from 57 out of the 320 traps), and 92 females and 9 males from sample 2 (originating from 47 out of the 320 traps) as follows. To each tube containing the legs of the tsetse, 200 μl of 5% Chelex® chelating resin was added (Walsh et al., 1991; Solano et al., 2000). After incubation at 56 °C for 1 h, DNA was denatured at 95 °C for 30 min. The tubes were then centrifuged at 12,000 × *g* for 2 min and frozen for later analysis.

The PCR reactions were carried out in a thermocycler (MJ Research, Cambridge, UK) in 50 μl final volume, using 10 μl of the supernatant from the extraction step. After PCR amplification, allele bands were routinely resolved on 10% non-denaturing polyacrylamide gels stained with ethidium bromide. To determine the different allele sizes, a large panel of about 30 size markers was used. These size markers had been previously generated by cloning alleles from individual tsetse flies into pGEM-T Easy Vector (Promega Corporation, Madison, WI, USA). Three clones of each allele were sequenced using the T7 primer and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA). Sequences were analysed on a PE Applied Biosystems 310 automatic DNA sequencer (PE Applied Biosystems) and the exact size of each cloned allele was determined. PCR products from these cloned alleles were run in the same acrylamide gel as the samples, allowing the allele size of the samples to be determined accurately.

Five microsatellite loci were used for genetic studies: *Gpg55.3* (Solano et al., 1997) which had been isolated from *G. p. gambiensis*, and *Pgp24*, *Pgp13*, *Pgp11*, and *Pgp1*, isolated from a laboratory colony of *G. p. palpalis* (Luna et al., 2001). At the time of this study these were the only loci available for tsetse, and they had been shown to be highly polymorphic and to show easily read banding patterns in laboratory-reared tsetse flies and in those from a preliminary sample from the field population being studied (data not shown).

The microsatellite locus *Gpg55.3* is on the X-chromosome (Solano et al., 2000; Gooding et al., 2004). Because of this and the predominance of females in the samples, we analysed mostly field-collected females.

2.4. Analysis of microsatellite data

For each population, Wright's F_{is} (within sample heterozygote deficiency, a measure of deviation from panmixia) and F_{st} (measure of population differentiation) were estimated using Weir and Cockerham's (1984) unbiased estimators (f for F_{is}, θ for F_{st}). These estimators were calculated with FSTAT V. 2.9.3 software (Goudet, 1995). For random mating (within

samples) or random distribution of individuals (between samples), F values are expected to be zero.

The significance of F_{is} (deviation from panmixia) was also tested for each locus, and for all loci, in each sample, and simultaneously in several samples, using 10,000 permutations of alleles between individuals in FSTAT. Males were hemizygous at locus *Gpg55.3*. For that locus, measure of F_{is} and its significance were conducted only on females. The significance of F_{st} (population differentiation) was assessed using 10,000 permutations of genotypes among samples (FSTAT). Linkage disequilibrium was tested by the exact test of GENEPOP 3.3 for genotypic linkage disequilibrium (Raymond and Rousset, 1995).

The frequency of null alleles that was necessary to explain our observed F_{is} was calculated following Brookfield's (1996) method. The comparison between the expected occurrence of null individuals (blanks) and the real blanks observed was undertaken by a unilateral exact binomial test under S-Plus 2000 professional release 1. Short allele dominance can be recognised by regressing the F_{is} of each allele against allele sizes (see Wattier et al., 1998; De Meeüs et al., 2002), we thus attempted to find such a pattern in the available data using S-Plus 2000 professional release 3. The basic assumption of the model is that in a heterozygous individual, the shortest allele will be better (or faster) amplified by a factor proportional to the size difference between the two alleles in concern. Then a Spearman correlation coefficient and its P-value were computed, together with R^2 (regression's coefficient of determination), this latter measuring the percentage of the F_{is} explained by allele size. As the relationship expected under the short allele dominance hypothesis is negative, we only kept the regressions giving such a relationship.

A Bayesian approach was used to explore the genetic structure of our samples, using Bayesian Analysis of Population Structure (BAPS 3) software (http://www.rni.helsinki.fi/~jic/bapspage.html) (Corander et al., in press). BAPS 3, a program for Bayesian inference of the genetic structure in a population, treats both the allele frequencies of the molecular markers and the number of genetically diverged groups in a population as random variables. It uses stochastic optimization to infer the posterior mode of the genetic structure. Goodness-of-fit levels of the results are compared in terms of the ''log ml-values'' (natural logarithm of the marginal likelihood of the data) provided for any particular clustering solution. As recommended by the authors, the program was run with several values of K. The first runs were set with $K = 2$ to $K = 30$, K being the potential maximum number of genetically different groups (which will be called ''clusters''), and was run five times for each value of K. For each K value (even the replicates of the same value) the program finds the optimal partitions with $k \leq K$. Then, because the best partitions seemed to lie somewhere below 30 we undertook many runs (at least 500) with $K = 30$. After having found the value of K which gave the highest ''log ml'' value, the F_{is} provided by this best partition was computed and its significance tested under FSTAT as above. The comparison between initial F_{is} (without partition) and the values obtained with the best partitions was also undertaken with a Wilcoxon signed rank test for paired data, the pairing criterion being the individual loci (five data for each sample). BAPS exploration with $K = 50$ tested 1000 times was also applied on the whole data set (November 2000 and January 2001 samples pooled) in order to confirm the partitions found and to detect possible common clusters between the two samples. The last point was also checked with a tree construction approach on Cavalli-Sforza and Edwards (1967) chord distance between the clusters defined by BAPS. This distance is indeed the most appropriate for tree construction (Takezaki and Nei, 1996). The distances were computed by the GENETIX V. 4 software package (Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France). The distance matrix obtained was then used to build a dendrogram (neighbor joining method) (Saitou and Nei, 1987) using MEGA Version 3.1 (Kumar et al., 2004).

The homogeneity of age distribution among samples and clusters was tested with an analysis of variance with the software S-Plus 2000 professional release 3. For that, the factor ''cluster'' was nested into the factor ''sample''. The influence of fly's age on the infection with trypanosomes was investigated through a logistic regression with S-Plus 2000. The initial model was of the form:

$$\text{Trypanosome presence} \sim \text{Sample} + \text{Age} + \text{Cluster (nested in sample)} + \text{Constant}.$$

A stepwise process allowed selecting for the minimum model and a Chi-square test was used to test the significance of the model (see S-PLUS, 2000 Guide to Statistics vol. 1 for more details). Comparisons of trypanosome prevalence between clusters in each of the two samples were done using Fisher's exact test (procedure STRUC in GENEPOP 3.3).

3. Results

3.1. Entomology

Of 7689 tsetse that were trapped (5664 females and 2025 males), all belonged to *G. p. palpalis*, based on morphological characters and on geographic distribution. Of these flies, 4421 were caught in November 2000 (sample 1, end of rainy season) and 3268 were trapped in January 2001 (sample 2, cold dry season). The apparent density averaged 3.45 tsetse/trap/day in the first survey, and 2.55 in the second, but varied between 1.4 and 8.5 according to the biotope. The sex ratio, 2.8 females to 1 male in the total sample, was typical of catches obtained with the Vavoua trap.

3.2. Epidemiological results

Among the 906 tsetse (799 females and 107 males) that were dissected, 25.06% were infected by trypanosomes. PCR techniques applied to these infected tsetse identified the following types of trypanosome infections: *Trypanosoma congolense* of the West African/Riverine forest type (*Tcf*, 6.5%), *Trypanosoma vivax* (*Tv*, 5.8%), *T. brucei* sensu lato (*Tb*,

Table 1
Characteristics of the five microsatellite loci used to study *G. p. palpalis* in Bonon, Côte d'Ivoire

Sample	Locus	Genic diversity	F_{is}	*P*-value	Frequency of most abundant allele	Number of alleles
1 (November 2000)	*Gpg55.3*	78.37	0.436	<0.001	0.44	17
	Pgp24	87.67	0.229	<0.001	0.26	20
	Pgp13	76.57	0.443	<0.001	0.42	16
	Pgp11	73.98	0.326	<0.001	0.4	10
	Pgp1	88.29	0.306	<0.001	0.23	14
2 (January 2001)	*Gpg55.3*	77.02	0.463	<0.001	0.44	17
	Pgp24	82.50	0.559	<0.001	0.30	13
	Pgp13	71.62	0.474	<0.001	0.44	13
	Pgp11	70.97	0.228	<0.001	0.47	11
	Pgp1	85.88	0.118	<0.001	0.29	16

2.6%), and *T. congolense* of the savannah type (*Tcs*, 0.70%). Among the tsetse infected with *T. brucei* s.l., only three individuals were infected with *T. b. gambiense* group 1, as indicated by the gambiense group 1 specific molecular marker. One of these flies had a salivary gland infection that was also detected by microscopy. Some of the trypanosomes observed by microscopy could not be identified by the molecular markers used (''undetermined'' trypanosomes).

The mean age of the 770 females, determined by ovarian dissection, was 34.5 days. Age composition of these flies was 1.8% teneral (i.e. recently emerged but not fed), 14.2% nulliparous (no offspring deposited), 39% young females, and 45% old (i.e. more than 40 days).

3.3. Polymorphism, heterozygosity, null alleles, and short allele dominance at microsatellite loci

The number of alleles, at each locus, varied between 10 and 20 within each sample, and genic diversity varied between 70 and 88% (Table 1). Weir and Cockerham (1984) estimators indicated large heterozygote deficiencies within each sample (over all loci $F_{is} = 0.344$, $P < 0.001$, and $F_{is} = 0.366$, $P < 0.001$, for samples 1 and 2, respectively), with values of F_{is} by locus ranging from +0.12 to +0.56. All five loci deviated from Hardy–Weinberg expectations, showing a much lower number of heterozygotes than expected. No linkage disequilibrium was found between any pair of loci in either sample (data not shown). To explain these highly significant heterozygote deficiencies, both technical (e.g. undetectable nature of null alleles) and biological (e.g. Wahlund effect or inbreeding) factors must be taken into account.

If locus *Gpg55.3* is located on the X-chromosome (Solano et al., 2000; Gooding et al., 2004), and if null alleles were the cause of the heterozygote deficiencies, we would have expected to find null males. However, all males showed one band. In sample 2, under Brookfield hypothesis (Brookfield, 1996) with a frequency of null allele $r = 0.19$, 1.6 null males were expected (out of 8), whereas all males showed one band. The binomial *P*-value was 0.17 which was not significant, but was the minimum possible value given the small sample size. Even in females, the frequency of null alleles accounting for the observed deficiency would be so high (0.2) that null homozygotes (i.e. females showing no PCR product) would have been expected (Brookfield, 1996), but none was observed. Thus, although the presence of null alleles could not be rejected, it is not likely that null alleles could explain the observed heterozygote deficiencies at this locus.

Primers were successfully redesigned for loci *Pgp24* and *Pgp13* (which showed the highest F_{is} values). However, we were not able to redesign efficient primers at the other loci. Using the new primers for locus *Pgp24* in sample 2, F_{is} decreased from +0.56 to +0.42, and at locus *Pgp13*, F_{is} decreased from +0.47 to +0.39. Despite this decrease in F_{is}, heterozygote deficiency remained significant at each locus (*Pgp24*, $F_{is} = 0.42$, $P < 0.0001$; *Pgp13*, $F_{is} = 0.39$, $P < 0.0001$).

There was a negative correlation between allele size and F_{is} value at four of the five loci (not at *Pgp11*), that is, high F_{is} values for short alleles, which can be called short allele dominance. The associated *P*-values appeared significant for both samples at locus *Pgp1*, and for either sample 1 or sample 2 at the three other loci (see Table 2). When the *P*-values were significant, they explained between 23 and 57% of the total variance of F_{is} at the locus.

We concluded that null alleles and/or short allele dominance may partly explain the overall heterozygote deficiency, but were not sufficient to explain it entirely, and that biological hypotheses had to be investigated.

Table 2
Results of regressions for each locus in each sample that showed a negative relationship between allele size and F_{is} value, as expected under the hypothesis of short allele dominance

Locus	Sample	Rho	*P*-value	R^2
Gpg55.3	1	−0.62	**0.01**	0.43
Pgp24	1	−0.69	**0.003**	0.57
Pgp13	1	−0.48	0.06	0.19
	2	−0.61	**0.03**	0.35
Pgp1	1	−0.66	**0.02**	0.43
	2	−0.54	**0.04**	0.34

Numbers in bold represent significant *P*-values (<0.05) associated to the Spearman correlation coefficient (see Section 2 for details). R^2 is the coefficient of determination of the regression and represents the percentage of variance of F_{is} that is explained by the size of alleles, and hence illustrates with what magnitude short allele dominance explains the heterozygote deficits.

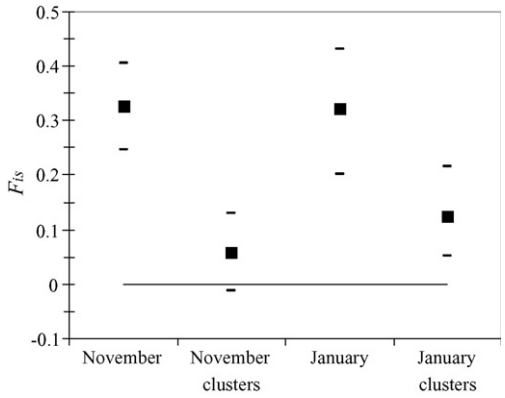

Fig. 2. F_{is} values estimated for both samples 1 and 2 *G. palpalis palpalis* before (November and January) and after BAPS partitioning of the data (clusters). 95% confidence intervals were obtained by bootstraping over loci (See Goudet, 1995).

3.4. Geography

Our samples allowed us to test whether a Wahlund effect has occurred through artificially grouping populations from different traps. To do this, tsetse were analysed according to the trap in which they were caught, considering each trap as a population. This was possible for 28 traps in which the number of females analysed was more than one. The overall F_{is} was 0.29 (0.22–0.38, 95% bootstrap confidence interval), showing no significant decrease compared to the pooled data ($F_{is} = 0.344$).

We also tried to group individual flies geographically, and we defined arbitrarily five zones: North, West, South, East, and the town of Bonon. Overall F_{is} was still 0.30 (0.24–0.38, 95% bootstrap confidence interval).

3.5. Identification of genetic groups within a sample

The best partitions obtained with the BAPS analysis provided 25 clusters in sample 1 and 21 clusters in sample 2. As shown in Fig. 2, the heterozygote deficiency dramatically dropped in both samples after clustering. In the partition obtained in sample 1, the F_{is} was not significantly different from zero (P-value = 0.07), but it remained significant in the partition obtained in sample 2 (P-value = 0.0001). In both

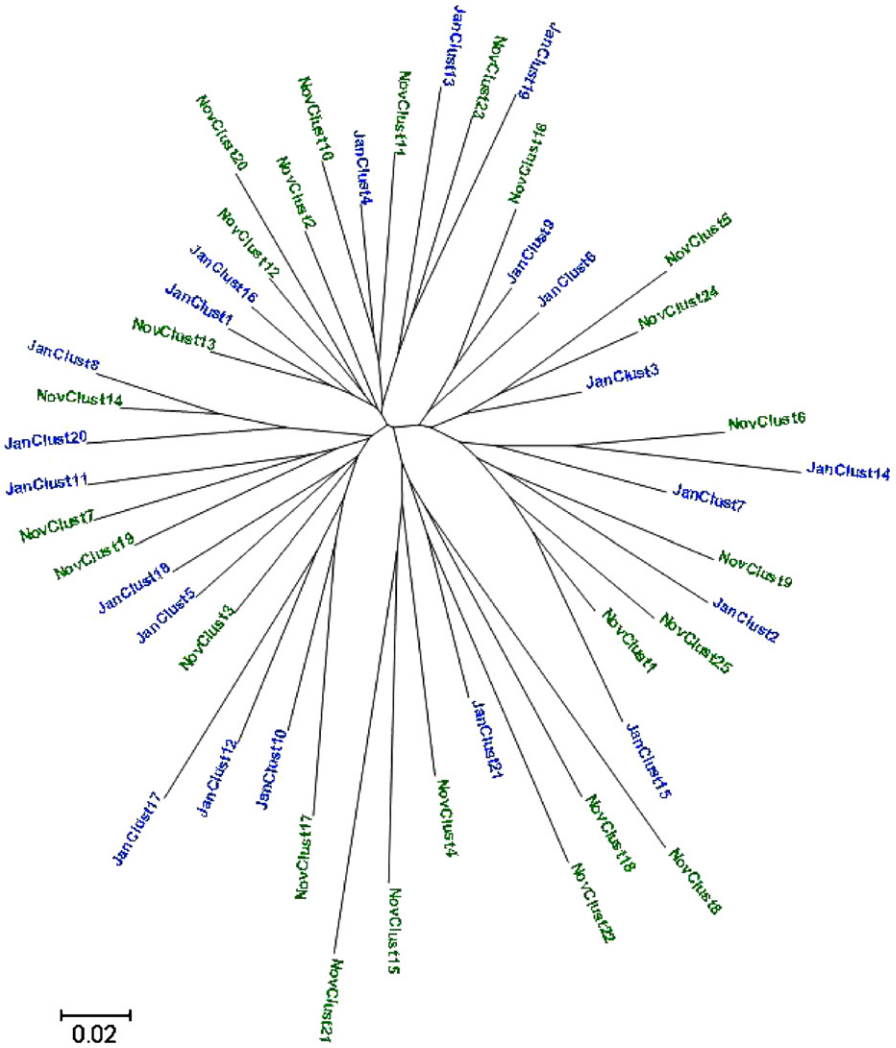

Fig. 3. Neighbour-joining tree obtained on Cavalli-Sforza and Edwards (1967) chord distances between BAPS clusters of *G. palpalis palpalis* from sample 1 (in green) and sample 2 (in blue).

samples the difference before and after clustering was significant (Wilcoxon signed rank test, P-value = 0.03).

The NJ tree obtained (Fig. 3) revealed no specific pattern, and except for rare pairs, no special relationship seemed to link samples 1 and 2 clusters. This was confirmed by the BAPS analysis undertaken on all individuals from both samples and pooled in a single one. The best partition obtained displayed 39 clusters. Less than one-third of these clusters (10) were heterogeneous with individuals from both samples, representing 46 individuals (among 225). This supports the lack of correspondence between clusters from samples 1 and 2. For both samples, the differentiation between clusters was $F_{st} = 0.29$ and 0.24 for samples 1 and 2, respectively, i.e. similar in magnitude.

3.6. Characteristics of the clusters

The composition of the 25 clusters of sample 1 and of the 21 clusters of sample 2 varied from 1 to 13 individuals per cluster, the mean being 5 for each of the two samples. Age was not significantly different between the two samples nor between clusters within samples (ANOVA, P-value > 0.16). The logistic regressions did not highlight any specific pattern except that more trypanosomes were found in sample 2 (prevalence = 0.41) than in sample 1 (prevalence = 0.29) (P-value = 0.023). Nevertheless, sample sizes were small and might have lowered the power of detection of some other patterns. Ignoring age, Fisher exact tests revealed that trypanosome prevalence (by microscopic examination) was heterogeneous among clusters from sample 1 (P-value = 0.001), but not from sample 2 (P-value = 0.169). Indeed some clusters had no trypanosome infections, or very low infection rates (e.g. cluster 3 from sample 1 and cluster 13 from sample 2), whereas others showed very high infection rates (above 40%, e.g. clusters 7 and 15 of sample 1 containing 13 individuals each, and clusters 3, 6, and 8 from sample 2 containing 7, 9, and 10 individuals each, respectively). Some of the trypanosomes found by microscopy could not be identified and were reported as ''undetermined''. With molecular determination of trypanosome species, because of small sample sizes, statistical testing was rarely possible. Some clusters harboured only one trypanosome type as recognised by PCR (e.g. clusters 8 and 9 from sample 1 and cluster 7 from sample 2), whereas others were shown to be infected by several trypanosome species or groups. For *T. brucei* s.l. the heterogeneity of prevalence was marginally significant in sample 2 (P-value = 0.042). For *T. congolense* of the Riverine Forest type, the distribution seemed homogeneous among clusters (P-value > 0.7 in both samples). For *T. vivax*, prevalence was heterogeneous only in sample 1 (P-value = 0.036) but not in sample 2 (P-value = 0.93). For the undetermined trypanosomes, no differences were observed in any sample (P-value > 0.6). Bloodmeal sources of some tsetse could be identified as coming from animals, e.g. clusters 8, 12, and 25 from sample 1, and cluster 3 from sample 2. All those clusters having such identified bloodmeal sources showed trypanosome infections.

4. Discussion

The present study was undertaken to gain knowledge of the population structure of *G. p. palpalis*, the main tsetse species found at Bonon, a recently described, very active HAT focus in west-central Côte d'Ivoire. The rationale for this work is the assumption that a knowledge of the genetic structure of a vector population will help provide a sound basis for understanding an epidemic and will contribute to a rational control operation, especially one that involves a genetic approach to vector control. Allele frequencies were determined at five microsatellite loci in two temporal samples. Highly significant heterozygote deficits, i.e. strongly positive values of F_{is}, the within-population index measuring deviation from random mating, were observed at the five microsatellite loci in each of the two samples taken three months apart.

These high F_{is} values were not expected and are not usual values found in other Dipteran groups. However, molecular data on tsetse have been very scarce up to now, and whenever they exist they are mostly directed towards *morsitans* group species. For instance, in the review of Gooding and Krafsur (2005), there is basically random mating within populations but not among (in *G. pallidipes* and *G. morsitans*), which was found surprising by the authors compared to the reported vagility of tsetse. The first preliminary study on natural populations of *G. palpalis gambiensis* in Burkina Faso using microsatellite markers showed high F_{is} values which were explained by the existence of a Wahlund effect (Solano et al., 2000). These authors did not find so high F_{is} values in other localities, for instance in Senegal and in another area of Burkina Faso (Solano et al., 1999), where there was no indication of deviation from within population random mating. In one of the few studies on tsetse using microsatellite DNA loci (Luna et al., 2001) where they developed 13 microsatellite loci and tested them on several lab reared tsetse taxa, the only field population of *G. palpalis gambiensis* they tested (coming from Burkina Faso, 30 individuals) showed heterozygote deficiency at 11 out of 12 loci, with 3 loci showing significant departure from H–W expectations. Elsen et al. (1994), using multilocus enzyme electrophoresis on laboratory-reared *G. palpalis gambiensis*, also found departure from Hardy–Weinberg expectations due to significant heterozygote deficiencies at some of the loci studied.

The situation here may also be complicated by the fact that the study area is located close to the northern limit of *G. p. palpalis*, hence to the limit between the two subspecies of *G. palpalis* (Challier et al., 1983). The possibility cannot be ruled out that some of the clusters contain individuals coming from crosses of the two subspecies, although in the laboratory hybridisation between these subspecies leads to sterile males and fertile females (Southern, 1980; Gooding, 1997), and thus females provide a bridge for introgression of genes. The possibility that *G. palpalis* indeed represents a species complex should be examined on a wider geographic scale.

In the present study, null alleles were demonstrated at two loci by using redesigned primers, but their frequency was too low to fully explain the heterozygote deficits. Short allele

dominance was also observed and accounted probably for part of the total F_{is}. Geographic structuring did not explain the observed deficiencies, regardless of the geographic scale used in the analysis. Even at the scale of a single trap, for some of the traps, heterozygote deficiencies remained very high and statistically significant.

The significant decrease in heterozygote deficits, leading eventually to a non-significant value in sample 1, and observed also in at least 39 of the clusters that were defined by the BAPS approach, strongly suggests that the Vavoua traps attracted tsetse flies from numerous, strongly differentiated units (i.e. sub-populations or groups). The initially high heterozygote deficits are thus most likely explained by a strong Wahlund effect and, to a lesser extent, by the presence of null alleles and short allele dominance. These tsetse groups would be of small size, which is generally acknowledged as being typical for tsetse (e.g. Gooding and Krafsur, 2005), and would favour genetic differentiation due to genetic drift. Tsetse flies may be vagile enough (see Leak, 1999 for review) to enable the trapping system to sample many of these genetically differentiated groups, leading repeatedly to high F_{is} values. Genetic drift is not necessarily contradicted by the reported vagility of *G. palpalis* since tsetse may not use their maximal capacities of dispersal if they do not need to: very recent data on mark-release-recaptures of *G. palpalis* in Burkina Faso showed that when tsetse are released in very favorable area (i.e. an area with humidity and shade typical of *G. palpalis*), a statistically significant number stays within this area, whereas when they are released in a degraded area, they tend to look for a favorable area and then diffuse on much longer distances (J. Bouyer, personal communication). The Wahlund effect found here then reveals the artificial grouping in the traps of several different genetic groups. In addition, most of the traps being placed only on sites daily frequented by HAT patients, they are likely to catch mostly tsetse that are looking for a bloodmeal. If there is sub-structuring, tsetse from different subgroups would be artificially mixed in these traps when they come to hunt. If traps were placed near larviposition sites, in more conserved areas, F_{is} values might be lower, and that is a possibility that will deserve further work. Depending on availability of hosts in the vicinity of these different subgroups, the different clusters would appear to be differentially infected, as may be the case in this study. It is not known whether these genetically different populations differ in their vectorial competence and/or host preferences, as previously suggested with other field-caught tsetse (Solano et al., 2000) and in laboratory experiments (Elsen et al., 1994).

The fact that little correspondence was found between the clusters defined in the two samples suggests that, because of the number of groups and the number of traps from which the samples originated (57 for sample 1, 47 from sample 2, 14 of which were exactly at the same place), the probability of catching individuals from the same sub-population during two temporally distinct sampling periods was very low. Possible exceptions include cases where traps were placed at precisely the same locations in both sampling periods and attracted flies from habitats that supported the same sub-populations, despite any microhabitat changes that may have occurred between sampling dates. It has also to be said that the number of clusters found here does not necessarily represent the true number of existing sub-populations, because this will depend very much on the spatial distribution of the traps within the area together with the dispersal capacity of the tsetse, and also because the number of BAPS clusters might have considerably been increased by short allele dominance at several loci.

Our suggestion that the *G. p. palpalis* population at Bonon composed of small units is consistent with the reported vulnerability of tsetse to trapping, which fortunately, is an efficient control method (Laveissière and Penchenier, 2005; Kuzoe and Schofield, 2005). The suggested population structure may also account for both the reported ability of tsetse populations to recover rapidly from low population densities and for their ability to rapidly re-invade controlled areas (Torr et al., 2005). This result also suggests that traps may result in a sampling bias, and that other sampling methods (e.g. Muzari and Hargrove, 2005) should be considered in order to look for the real reproductive units. Finally, the genetic pattern observed in *G. palpalis* populations may be explained either by very small sub-population sizes and/or by a strong phylopatric behaviour, as larval pheromones have been suspected to occur (Nash et al., 1976; Gouteux et al., 1983).

Since the HAT focus of Bonon has been only recently described, and since HAT displays an epidemic pattern at Bonon (Djè et al., 2002), an understanding of the dynamics and composition of the local tsetse populations is undoubtedly a crucial issue. Such knowledge may elucidate the importance of HAT, which seems to be directly linked to human and environmental changes in this area (Courtin et al., 2005). Studies on both subspecies of *G. palpalis* s.l. (Solano et al., 2000 and the present one) concluded that the presence of a Wahlund effect explains, at least in part, the genetic structure of natural populations of this species. Future work ought to design other sampling strategies to access the real reproductive units (sub-populations) of this important vector species. Furthermore, future work needs to examine additional molecular and morphometric markers on the same individual to shed light on this issue. Finally there is a need to extend these studies to other tsetse species in other geographic locations to help determine on where and how to eliminate tsetse.

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Review

Sleeping sickness in West Africa (1906–2006): changes in spatial repartition and lessons from the past

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Summary

OBJECTIVE To review the geography and history of sleeping sickness (Human African trypanosomiasis; HAT) over the past 100 years in West Africa, to identify priority areas for sleeping sickness surveillance and areas where HAT no longer seems active.

METHOD History and geography of HAT were summarized based on a review of old reports and recent publications and on recent results obtained from medical surveys conducted in West Africa up to 2006.

RESULTS/CONCLUSIONS Active HAT foci seem to have moved from the North to the South. Endemic HAT presently appears to be limited to areas where annual rainfall exceeds 1200 mm, although the reasons for this remain unknown. There has also been a shift towards the south of the isohyets and of the northern distribution limit of tsetse. Currently, the most severely affected countries are Guinea and Ivory Coast, whereas the northern countries seem less affected. However, many parts of West Africa still lack information on HAT and remain to be investigated. Of particular interest are the consequences of the recent political crisis in Ivory Coast and the resulting massive population movements, given the possible consequences on HAT in neighbouring countries.

keywords sleeping sickness, West Africa, northern limit, migration, population movements, rainfall

Introduction

Human African trypanosomiasis (HAT) appeared in cyclical epidemics on the African continent in the 20th century. After an epidemic from 1900 to 1950, HAT was considered under control in the 1960s as a result of a vast campaign based on active screening and treatment of the patients by mobile medical units (Jamot 1935; Richet 1962). In the 1970s, the number of cases gradually increased again and over the last 20 years the situation has again become as alarming as it was 100 years ago.

In 2000, the estimated number of infected individuals was approximately 500 000 (Cattand *et al.* 2001). Over the last 5 years, screening – by mobile units – and treatment have greatly increased in Central Africa, the region most affected by HAT. The results seem promising since in 2004 only 17 600 cases were reported (WHO 2006). Disease elimination may be considered to be underway (Jannin 2005), although this process may take a long time since only 10% of the 60 million people at risk are under surveillance. For instance, in West Africa, no reliable information is available on how widespread HAT is in the historical foci of Guinea, Ivory Coast, Burkina Faso, or Liberia, Sierra Leone and Ghana.

Human African trypanosomiasis typically develops in geographically limited foci, which implies that interactions between vector (tsetse fly), parasite (trypanosome) and host (human as well as animal) are complex and influenced by the biophysical and human environment. Efforts to control this disease – beyond the knowledge on the parasite/vector/host complex – need to take into account more global data considering economic, social and climatic factors.

The history of HAT has already been discussed in a variety of publications, on different scales, for Africa

(Louis *et al.* 2002), Central Africa (Penchenier *et al.* 1996) and East Africa (Hide 1999). However to our knowledge, these reports do not cover how HAT has evolved geographically in West Africa in the last 100 years, although a great number of epidemiologic data are available thanks to reports of surveys performed by medical teams. This study – in no way exhaustive – of available HAT data, combined with the field experience of the authors of the present article, attempts to trace back how this pathology evolved over time and space in the sub-region and to correlate it with the factors which may play a significant role in how sleeping sickness foci evolved geographically, such as human migrations and climate (annual rainfall). Their impact on the environment and on the distribution of the tsetse seems to play a significant role on the observed spatial changes of sleeping sickness distribution since last century.

Material and methods

This study collected spatialized data on HAT foci, organization of colonial health services, and human (mainly migration) and environmental factors in West Africa, as well as northern tsetse ditribution limit, from the beginning of the 20th century to the present time. A number of libraries were consulted: Institut de Médecine Tropicale du Service de Santé des Armées (IMTSSA) Marseille, France; Centre d'Archives d'Outre-Mer (CAOM), Aix en Provence, France; Institut de Recherche pour le Développement (IRD), Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Paul Valéry University, Montpellier, France; Organisation Ouest Africaine de la Santé (OOAS), IRD, Centre International de Recherche Développement sur l'Elevage en zone Sub-humide (CIRDES), Bobo-Dioulasso, Burkina Faso; IRD, Ouagadougou, Burkina Faso; IRD, Abidjan, Ivory Coast.

Contemporary data were collected from bibliographies (articles and mission reports) and field surveys that the authors of this article have contributed to in Ivory Coast, Guinea, Mali, Benin and Burkina Faso. These medical surveys were all conducted by national programme teams with the technical support of Institut Pierre Richet (Ivory Coast) and the Institut de Recherche pour le Développement (IRD), with most of the support from World Health Organisation (WHO) and French and Belgian cooperation agencies.

Results

Population movements and HAT at the beginning of the 20th century (1900–1930)

At the beginning of last century, according to old reports sleeping sickness was highly prevalent in large areas of Guinea and former Upper Volta (now Burkina Faso), going up in latitudes to the Gambia and Casamance, but also to Senegal and Niger rivers up to Saint Louis in Senegal, and Timbuctu in Mali, even though most of the cases of these 'foci' were probably imported from elsewhere due to population movements. The disease was also present in Ivory Coast, Ghana and Benin (Figure 1).

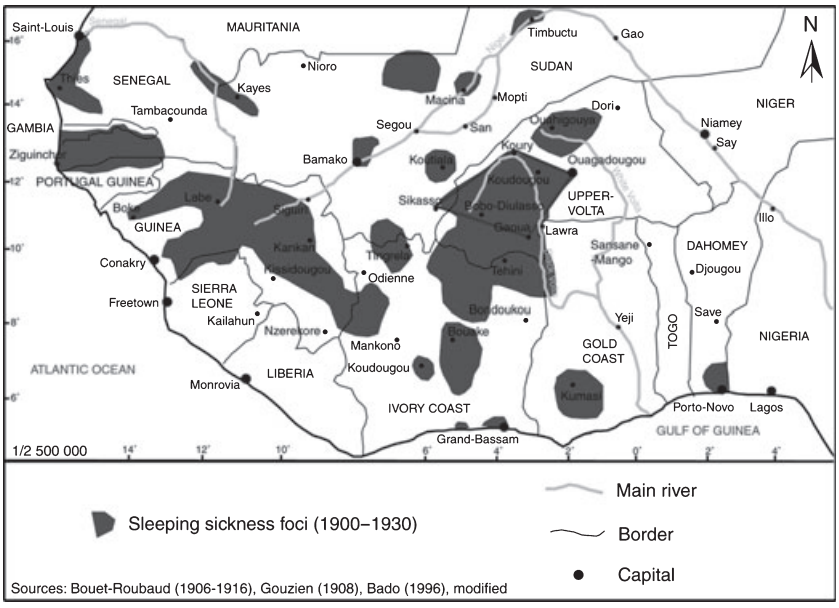

Figure 1 The distribution of West African sleeping sickness foci in the early 20th century.

Between 1906 and 1908, Paul Gouzien, then head of Upper Senegal and Niger (i.e. currently Mali, Burkina Faso and Niger) Health Service, wrote a report on the disease situation, in which he pointed out that 'the endemic disease seems to be essentially confined within the limits of a quadrilateral formed by Koury, Ouagadougou, Gaoua (Burkina-Faso) and Sikasso (Mali)' (Figure 1). Gouzien (1908) thought that 'the endemic–epidemic first seems to have gone up the Volta river right from its estuary in the gulf of Guinea, spreading afterwards inland through the network of its numerous tributaries'. The commercial relations that the Ashanti kingdom in the Gold Coast (present-day Ghana) had with the Sahelian zone, as well as the advance of the English-occupied hinterland towards the north (M'bokolo 2004) suggested that the disease actually spread from the coast up into the interior of these countries, essentially from Kumasi focus. Samory Touré, who started his riding feats from Kankan (Binger 1982) where HAT was already present (Figure 1) also seem to have played a role in the spread of HAT towards northern Ivory Coast and southern Burkina Faso. For example, in Gaoua, South of Burkina Faso, 'the inhabitants of Danhalle, Laussera and Diodiona are very positive about that and declare the disease was not known before the Lobi invasion by Samory' (Gouzien 1908). The descriptions of certain places in this report reveal the impact of the disease on settlement: 'As for Kaho, a formerly beautiful village of 800–1000 inhabitants, located 400 m from the river, it is now completely wiped out: a palm tree in the middle of tumbling walls, and that is all. The last sick individual died in October 1906 and at the same time the only survivor took refuge at Kosso'.

After the First World War (1914–1918), France experienced a substantial decrease in population and viewed its colonies as a source of raw materials and labour, even though these populations were threatened with increasing morbidity and mortality. The French authorities then decided to control diseases in French West Africa by developing *l'Assistance Médicale Indigène* (Bado 1996). However, behind the will to improve Africans' health, there was the economic interest of French private and public companies. In reality, there were forced migrations of northern populations towards the various public works in French West Africa – the Thies-Kayes railways and the Ivory Coast railways between Bouake and Bobo-Diulasso, for example, which may explain why HAT was also found in these regions (Figure 1) – and into the forestry and agricultural companies in Ivory Coast. When Albert Londres came to West Africa, he wrote: 'So we come to Upper Volta in the Mossi region that is known in Africa under the name of 'men's reservoir': three million negroes. Everybody comes here to get men like water from a well. During the building of the Thies-Kayes and Kayes-Niger railroad, the Mossi region was tapped. The foresters came up from the lagoon and tapped the Mossi' (Londres 1929).

The French colonial policy thus also seems to have contributed to the spread of parasites from North to South, from the Upper Volta (which has now become Burkina Faso) to Ivory Coast and Ghana. First, this resulted from resettlement projects moving large numbers of labourers from Burkina Faso to Ivory Coast (for instance, Ivory Coast colonization villages were given the names of the original Mossi village of Burkina Faso e.g. Koudougou, Figure 1). In these villages, a medical examination every 2 week was compulsory and the trypanosome carriers were sent back to their Burkinabe villages, according to the rules applied at that time (Mandé 1997). Secondly, the policy prompted numerous Voltans to seek refuge in Gold Coast (present-day Ghana), where the English applied a more liberal policy. Thus in Ghana, among the patients screened from 1927 to 1931, nearly 200 had HAT at the Yeji post, in the northern territories, showing the close relation between the Voltan and Ghanean foci (Vaucel 1962).

From these early observations and field results at the beginning of the 20th century from Pasteur scientists, people were convinced of the importance of the endemic trypanosomiasis, and above all of the epidemic form it took at that time in many regions (Dozon 1985), due in part to the numerous (forced) movements of people, especially between Burkina Faso, Ghana and Ivory Coast.

Colonial doctors, the first disease control programmes and HAT (1930–1950)

Following these observations, General Governor Brévié decided to create the Service de prophylaxie de la maladie du sommeil (the Sleeping Sickness Treatment Service) in 1931. Eugène Jamot travelled across the Upper Volta (Burkina Faso) and many regions of Ivory Coast, Sudan (now Mali), Guinea, Niger, Dahomey (now Benin) and Senegal (Jamot 1935) and, as well as Dr Gaston Muraz a few years later, confirmed that sleeping sickness was at that time the major public health problem of the colonies.

Figure 2 shows that the northern sleeping sickness areas described above already begin to shift to the South, except in Senegal. The Koudougou, Gaoua, Ouagadougou and Dedougou circles of Burkina Faso were still the most severely affected, together with Tougan sub-division of Dedougou, bordering the Black Volta (Mouhoun) river, where 20 years before the Koury post had been decimated by HAT. 'In the region of Gaoua morbidity and mortality rates caused by trypanosomiasis are impressive. We are not just talking about a few cases treated in Diebougou free

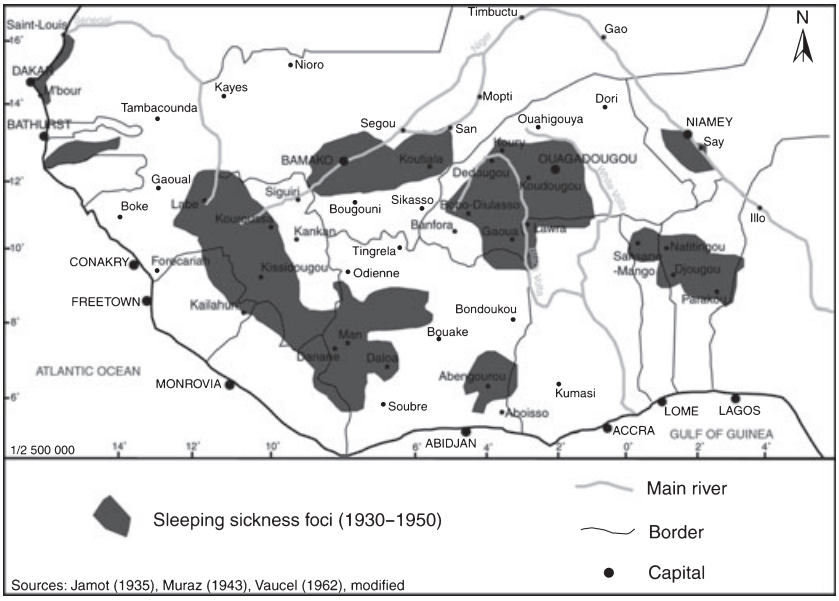

Figure 2 The distribution of West African sleeping sickness foci from 1930 to 1950.

Table 1 Number of HAT patients in West Africa

Country	1932–1934	1939	1940–1953	1961	1990–2004
Reference	Jamot 1935; Scott 1960	Muraz 1943; Scott 1960; Vaucel 1962	Masséguin & Taillefer-Grimaldi 1954; Vaucel 1962	Richet 1962; Vaucel 1962; Thomson 1968	WHO 2006
Senegal	54	2734	25 328	104	0
Sudan (Mali)	2580	8296	17 672	1151	78
Niger	369	1053	452	0	0
Haute Côte d'Ivoire Haute-Volta (Burkina Faso)	36 933	89 827	42 175	627	188
Dahomey (Benin)	6351	10 888	8588	86	189
Togo	*	*	*	71	5
Basse Côte d'Ivoire (Côte d'Ivoire)	1491	19 697	53 264	2680	3080
Guinea	4002	19 334	85 659	865	865
Sierra Leone	*	9,127	*	*	0
Liberia	*	*	12 768	*	0
Ghana	2945	4578	*	455	27
Nigeria	*	84 364	*	189	146
Total	49 336	249 898	245 906	6228	4578

*No information available to our knowledge.

clinic but about whole villages in the bush abandoned by their inhabitants in order to flee the disease' (Jamot 1933).

In Senegal, the disease was still active next to Dakar and around M'Bour (Jamot 1933; Figure 2). In Sudan (Mali), the disease raged between Bamako and Koutiala. In Niger, most of the sedentary villages South to Say were infected. In Dahomey (now Benin), Djougou and Natitingou constituted important border foci in relation with Sansane-Mango in Togo. In Guinea, the disease was present in Labe and Kissidougou. In Ivory Coast, the disease did not seem very active at that time, possibly because most medical screening was carried out in the northern countries, the main labour force reservoirs. However, the discovery of a large focus in Daloa showed that the disease was indeed present.

In the report of Jamot published in 1935 (Table 1), he concluded 'So, the number of sleeping sickness patients found in the French West Africa over a 2.5-year period,

with very limited means, was 45 238 on 1st October and it is of utmost interest to mention that this figure corresponds to the total number of patients who were shown in 1932 in the statistics for French Equatorial Africa (i.e. Central Africa), which is considered the sleeping sickness epicentre and where control measures have been carried out since 1917'.

In English-speaking colonies, the situation was also worrisome. In the border region of northern Liberia an epidemic occurred in 1938 among members of the Kissi tribe. For the members of this tribe living in French Guinea treatment had been available since 1930. From March 1941 to 1943 the first systematic survey was carried out by Veach (Firestone plantations) in the Kissi and Gbandi chiefdoms close to the Guinean border: 12 768 out of 81 915 people examined were sick (15% of the population) – 8000 of them belonged to the Kissi tribe (Vaucel 1962; see Table 1). In Ghana, in 1932 the increasing number of sleeping sickness cases became worrisome. The infection manifested itself above all in the eastern area of the Northern Territories and Mamprussi southern district where, from 1933 to 1936, 250, then 1012, 1683 and 2323 patients were detected; in addition, in the North-West area of the same territories, the Tumu/Lawra region had 2255 cases detected from 1934 to 1936 (Scott 1960).

In spite of Jamot's results, and the echoes of the disease in the neighbouring English-speaking colonies, medical inspector Louis Couvy abolished prophylaxis and trypanosomiasis services in 1935. It was not until 1939 that Gaston Muraz was charged with organizing the Service Général Autonome de la Maladie du Sommeil (SGAMS, Sleeping Sickness General Autonomous Service) in French West Africa. He divided French West Africa into sectors according to disease prevalence (highly affected, moderately affected or unaffected regions). This strategy enabled him to evaluate the situation for the first time, and in 1939, the number of sleeping sickness cases was 151 829 for French West Africa, and 249 898 in all of West Africa (Table 1).

Although three of the most affected sectors (Danane, Man and Daloa) were situated in Ivory Coast (Figure 2), sectors in Burkina Faso (also called Haute Côte d'Ivoire between 1932 and 1947, in contrast with Basse Côte d'Ivoire for Ivory Coast) were given greater importance in contemporary documents (Table 1). This contradiction stems from the fact that the disease had been known for a long time in Upper Volta, endemic areas were very extensive, and it was also a labour force reservoir area and therefore it appeared more urgent to control the disease there. In Ivory Coast, land clearing for wood industry and farming, essentially coffee and cocoa plantations, was made possible thanks to a considerable labour force from Upper Volta, which was contaminated because the disease was not controlled there (Kaboré 1957). For example, in 1938, the number of labourers under permanent contracts on the plantations was estimated at about 25 000 (Domergue-Cloarec 1986). Contract labourers were required to undergo a medical examination but many evaded it.

In 1944, SGAMS was remplaced by the Service Général d'Hygiène Mobile et de Prophylaxie (SGHMP, General Service of Mobil Hygiene and Prophylaxis), a new structure that had to treat endemic pathology (trypanosomiasis, onchocerciasis, malaria, leprosy). Bobo-Diulasso was still the center of the service, and Le Rouzic became the first director.

HAT just before and after independence (1950–1970)

The total number of sleeping sickness patients detected in French West Africa had reached 373 012 on 1st January 1954. Indeed, after some rather dispersed attempts, control had become better organized and more efficient after 1939. Just before independence HAT in the northern part of French West Africa seemed under control, except of a few active foci in the Gambia, Mali (San), the Lobi territory of Burkina Faso (Gaoua, Kampti, Tehini) and northern Benin (Figure 3). It still lingered in the forest zone, essentially in Guinea (Boke, Labe, Kissidougou) and Ivory Coast (Man/Danané and around Daloa in the Center West, and Abengourou in the South East), where it receded quite slowly (Masséguin & Taillefer-Grimaldi 1954).

Immediately after independence, HAT seemed under control in West Africa, it was then called residual trypanosomiasis (Richet 1962). At that time, Pierre Richet was in charge of controlling HAT, leprosy and onchocerciasis within the OCCGE (Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies/Organisation of Coordination and Cooperation for the control against Great Endemics).

In Upper Volta, 627 new cases were detected in 1961 but at least half of these cases had been contracted outside Upper Volta and involved seasonal agricultural labourers, most of them coming from Ivory Coast. However, a few Voltan foci remained, including Dedougou and Sindou with 80 new patients in 1961 in the village of Kankalaba. Since Guinea was not included in the OCCGE, data from this country were scarce and unreliable but the situation looked alarming notably because of a high rate of migration between the neighbouring zones of Guinea, Liberia and Sierra Leone (Hutchinson *et al.* 1964).

In Sierra Leone and Liberia, HAT was no longer considered a public health problem, and the Endemic Diseases Control Unit activity was more oriented towards malaria, onchocerciasis, leprosy, yaws and tuberculosis. In

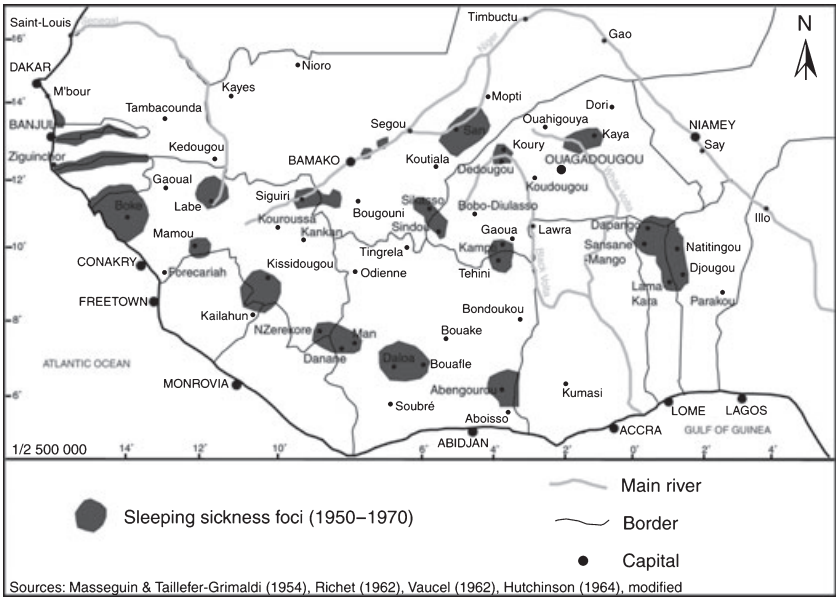

Figure 3 The distribution of West African sleeping sickness foci from 1950 to 1970.

Nigeria, the West African Institute for Trypanosomiasis Research controlled the disease in Benoue province, but the Eastern region still seemed to harbour the disease (80 new HAT patients in 1961) (Thomson 1968).

In the mid-1960s, the situation seemed under control to such an extent that Pierre Richet, in his introductory speech for the first international course on trypanology, said in his conclusion: "You must keep in mind, gentlemen, that HAT is an exceptional disease, that if it is neglected, or forgotten, it can turn into the horrid fire that nearly burnt down your Africa and its tribes during this century, that danger of revivescence will persist as long as there are *Trypanosoma gambiense* and tsetse flies. Those who have not lived recent and yet bygone past have no idea of the danger because it has become very difficult today to show them a classical HAT patient, a beautiful demonstrative 'sleeping' case" (Richet 1964).

HAT being neglected and a rapidly changing environment (1970–2006)

The impression that sleeping sickness had been overcome definitively, the disorganization of surveillance structures – stationary or mobile – but probably also changes in and new interactions between epidemiological parameters have enabled HAT to progressively re-emerge in West Africa since the 1970s, to reach back the impressive number of 500 000 cases estimated by WHO in 2001 for the whole Africa (Cattand *et al.* 2001).

In English-speaking West African countries, little information is currently available. According to WHO, in Ghana 27 cases have been diagnosed since 1990 (Table 1). In Nigeria 196 new cases were diagnosed in 1975 and 126 in 1976, and since the 1980s, some cases have been reported from the Delta State (Edeghere *et al.* 1989). No information on Liberia and Sierra Leone is available to our knowledge. In the French-speaking new independent states of West Africa, the fear for the reactivation of HAT foci seemed legitimate after the re-emergence of the Ouelessebougou focus in Mali in 1973 (Duvallet & Saliou 1976) and the Bouafle focus (Ivory Coast) in 1975 (Carrié *et al.* 1980). In Ivory Coast, the Centre-West region provided the largest number of patients, first with the discovery of the Vavoua focus in 1976 (Duvallet *et al.* 1978). Then came the Daloa and Zoukougbeu foci, the Sinfra focus with more than 4000 HAT patients between 1980 and 2000 (Laveissière *et al.* 2003) and finally today the Bonon focus (Djé *et al.* 2002; Solano *et al.* 2003), to which the Aboisso region near the Ghanean border must be added (Figure 4).

Since 2000 in West Africa, both Guinea and Ivory Coast are the most seriously affected countries (Figure 4). In Guinea, the coastal region had the best monitoring: there prevalences reach 1–3% in the mangrove foci of Dubreka, Boffa (Camara *et al.* 2005) and probably Forecariah, with sick individuals coming also from Sierra Leone (M. Camara, personal commmunication).

Looking at the current geographical distribution of HAT foci (Figure 4), the 1200 mm annual rainfall isohyet seems to constitute a northern limit of endemic HAT, with no cases having been found North of this isohyet since several years by active medical surveys, except some cases in Burkina Faso, Mali or Benin, most of which were imported

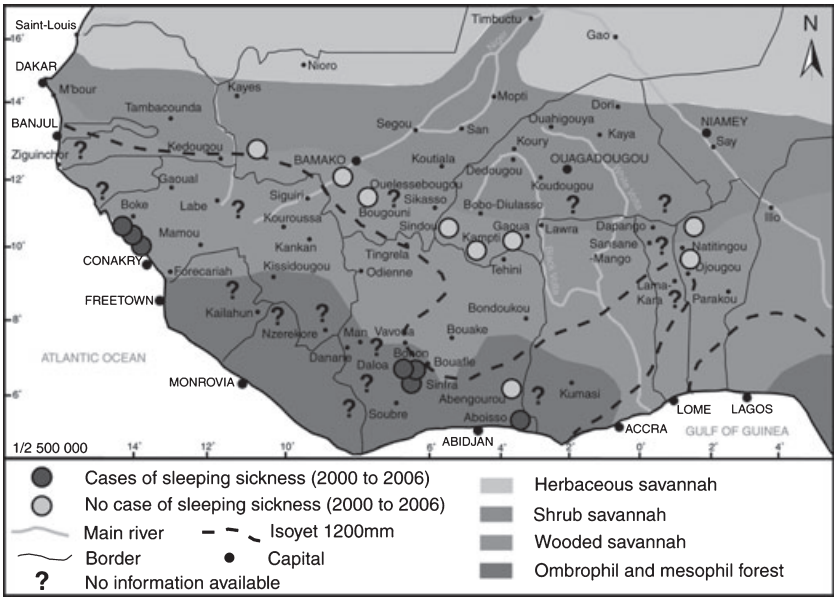

Figure 4 The current situation of sleeping sickness in West Africa according to medical surveys carried out between 2000 and 2006.

from Côte d'Ivoire (R. Kambire, personal commmunication for Burkina Faso) or were isolated cases in a non-endemic region. In Mali and Togo, the few recent medical surveys turned up no sick individuals in historical foci. The disease needs to be monitored regularly due to the small number of active medical surveys undertaken.

Looking more closely at climatic variations, it appears that rainfall series between 1951–1969 and 1970–1989 show a shift towards the South of the 500 and 1400 mm isohyets (Mahé & Olivry 1991; see Figure 5). The same shift towards the South is observed with the northern tsetse distribution limit, comparing catches of *Glossina palpalis* and *G. tachinoides* between the beginning of last century and present periods (Figure 5).

Discussion

In this paper, the historical information gathered on the geographical repartition of sleeping sickness foci in West Africa since last century, together with some information on climatic variation and evolution of tsetse distribution limits allows us to see some significant spatial changes that have occurred. The analysis of the historical records of sleeping sickness demonstrates that several human and physical elements play a major role in how the sleeping sickness foci evolve (spread or extinction, endemic or epidemic situation).

One element is the migration of farmers to new areas and their changing of the environment. A perfect example is the correlation over time and space in Ivory Coast between pioneering fronts of coffee/cocoa plantation (Chauveau 1985; Balac 2001) and HAT foci outbreak: HAT foci usually appear a few years after the successive waves of Voltan migrations required to clear the forest and set up and exploit these plantations (Rémy 1982; Laveissière & Hervouët 1988). However, these environmental changes beyond a certain extent of anthropization work against the maintenance of HAT foci, because the biotope favourable to *G. palpalis* HAT vector disappears (Laveissière & Meda 1999; Reid *et al.* 2000). This might explain the disappearance of HAT in zones where human density and pressure on the environment has become too great.

Conflicts entailing population displacements constitute the second element; Samory Touré's historical example being the most illustrative of this situation. Indeed, in the literature, conflicts are reported to encourage the development of disease (Prothero 1994; Kalipeni & Oppong 1998), including HAT (Prothero 1963; Eouzan 1980; Ford 2007), mainly because of health service disorganization and population displacement. The events of September 2002 in Ivory Coast may are a recent example of the impact of population displacements on the geographical changes of HAT (Kaba *et al.* 2006). These events resulted in the forced repatriation of 360 000 nationals from Burkina Faso (CONASUR, UNICEF, PAM 2004): will this situation trigger the re-emergence of HAT in Burkina Faso where tsetse flies are omnipresent in the south of the country? It is difficult to predict, but the question should be raised and answered, as it will probably enable better prediction of priority zones for HAT surveillance.

Element number three is border zone proximity. Examining old and recent reports and papers, it clearly appears

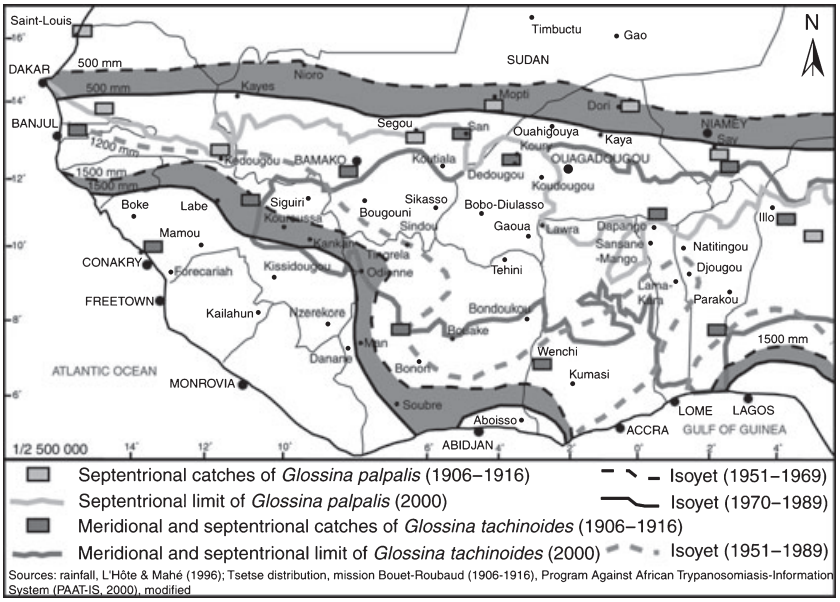

Figure 5 The North–South shift of the 500 and 1400 mm isohyets and of the tsetse northern distribution limit over time.

that border zones favour HAT development. One only needs to read through the list of cross-border HAT foci, be they historical or current ones: Danane-N'Zerekore (Ivory Coast/Guinea), Kampti-Tehini (Burkina Faso/Ivory Coast), Diebougou-Lawra (Burkina Faso/Ghana), Siguiri-Kangaba (Guinea/Mali), Guekedou-Kailahun (Guinea/Sierra Leone), etc. What is the reason for this? First of all, border territories often constitute sites of high levels of human mobility due to intense commercial activity. Secondly, they are often located far from the capital and therefore far from the main health infrastructures and health services. Finally, during troubles people seek refuge on the other side of the border; consequently at border areas, infected and susceptible people mix easily. Moreover, these border areas often constitute a favourable habitat for tsetse, as borders frequently follow rivers (Leraba, Mouhoun, Cavally, Mano, Senegal, etc.) and often also remain the last refuge for wild fauna (Taï, Bouna, Pendjari, Niokolo-Koba national parks), thus for tsetse which feed readily on these hosts.

Since last century endemic sleeping sickness has shifted from North to South. Most of the northern foci which produced the largest numbers of HAT cases during the last century are no longer active, i.e. Senegal, the Niger basin in Mali, the Volta foci in Burkina Faso, Niger. Now Guinea and Ivory Coast are the most affected countries, and even on the national level, the affected areas are coastal (littoral Guinea), or within the forest or forest–savannah transition zones (Centre-West Ivorian foci). Since the medical surveys conducted between 2000 and 2006 never found a confirmed HAT case in the historical foci situated above the 1200 mm isohyet, endemic foci in West Africa seem to be confined now to the zone below this isohyet. This observation does not mean that cases can not be found at these latitudes, but that endemicity seems now restricted to this limit, although we are not yet able to explain it.

Comparing the spatial changes that have occurred since last century elicits several questions: Why does the HAT decline seem to occur in Northern sub-Saharan Africa countries, such as Senegal, Mali, Burkina Faso and Niger, which provided tens of thousands of cases in the first half of the 20th century? Why do we have the impression that today HAT is limited to West African coastal and forested zones? Does the spatial distribution of the current foci, showing an absence of endemic HAT in regions with <1200 mm rain per year, fit in with the facts, or does it merely mirror the zone where medical teams monitor the disease effectively?

There is no doubt that human activities play a role in the appearance or disappearance of HAT foci (see above), but climatic changes – following the example of other pathologies (Amat-Roze 1998) – might play a part as well, although this may be difficult to separate from the role played by human populations. The steady decrease in rainfall between years 1950–1969 and 1970–1989 illustrated in Figure 5 has been said to be worsening these last few years (L'Hôte & Mahé 1996; Paturel *et al.* 1998). Indeed, the decrease in rainfall alters the biophysical – hygrometry, physionomy – characteristics (Mahé & Olivry 1991) of forests and gallery forests, the main tsetse fly

biotopes. The decrease in cereal yields (corn, sorghum, rice, etc.) consequent to this phenomenon, coupled with a rapid population growth, gives cause to more land cultivated and a simultaneous loss of tsetse habitat.

Linked to this shift towards the South of isohyets, the tsetse (*G. palpalis* and *G. tachinoides*) northern distribution limit has also shifted towards the South since the beginning of last century (PAAT-IS 2000; see also Figure 5). This has been clearly illustrated at local scales, e.g. in Togo for *G. palpalis* (Hendrickx *et al.* 1999). Therefore, the extinction of some historical foci, such as Saint-Louis in Senegal and Mopti in Mali, can be attributed to the local disappearance of the vector. However, many other factors than tsetse presence are required to explain the current geographical distribution of active foci. For instance, understanding why there is no more endemicity in Burkina Faso, although this country was the most seriously affected, and considering that tsetse are still present in the historical foci, may bring interesting elements for risk prediction and management.

Also, by looking back at Figures 1–4, one can see that there have been changes in the way of representing foci, from big, widespread spots at the beginning of last century, to small circles these last years. Does this signify a real decrease in sleeping sickness prevalence, or only a different way of considering a sleeping sickness focus?

However, the current pictures only reflect the few medical surveys that have been conducted, and much remains to be studied: what is the situation in South Burkina Faso taking into account the thousands of returnees who were living in endemic areas of Ivory Coast? Does the same apply to the Ivory Coast/Liberia/Sierra Leone/Guinea borders with the recent crises? What about Ghana? Sierra Leone? Liberia? the forest area of Guinea? etc. It already seems justified to prioritize surveillance in the most affected areas under the 1200 mm rainfall limit, but HAT surveillance must also continue in the humid savannah areas of Burkina Faso, Mali, and Benin, where information remains incomplete.

Conclusion

The analysis of the changes in HAT over space and time in West Africa enabled us to observe that nowadays, endemic HAT does not seem to occur any more in regions where annual rainfall is <1200 mm/year, which was not so at the beginning of 20th century. The decrease in rainfall, associated to increasing human densities, have an impact on land saturation, which has also changed tsetse distribution. These factors explain in part HAT geographical evolution (disappearance of northern historical foci which are nowadays situated North of tsetse presence).

Understanding how these factors interact with others, such as the link between HAT and population movements due to political crises, the decrease in numbers of wild animals, changes in vector competence or human–vector contact, will explain the geography of HAT foci and deserve further research. We remain convinced that to be sustainable, the elimination process recently launched by WHO has to be linked with substantial research focussed on the study of the conditions of emergence/re-emergence and extinction of the disease. An in-depth study of the spatial arrangement of these factors on the scale of West Africa will enable the prediction of intervention priority zones and will optimize control against HAT.

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Résumé:

Les glossines, ou mouches tsé-tsé, sont les vecteurs des Trypanosomoses Humaine (THA ou maladie du sommeil) et Animales (TAA) africaines, qui constituent encore un fardeau énorme au niveau santé publique et en terme de pertes économiques pour l'Afrique subsaharienne. Les travaux effectués depuis une dizaine d'années permettent maintenant de comprendre la structure des populations de *Glossina palpalis*, vecteur majeur des trypanosomoses en Afrique de l'Ouest, ainsi que les interactions spatiales entre groupes taxonomiques de trypanosomes et espèces de vecteur. *G. palpalis* s'organise sous forme de sous-populations vivant chacune dans un espace confiné, génétiquement distinctes, de petites tailles, échangeant un flux limité de gènes entre elles. Le degré de « fragmentation » de ces populations semble lui-même dépendre du degré de fragmentation de leur habitat, riverain ou forestier. Cette glossine s'adapte par ailleurs très bien à un habitat fortement urbanisé et est capable de se maintenir en faibles densités suite à des opérations de lutte, ce qui en fait un vecteur toujours redoutable malgré l'explosion démographique africaine et les changements climatiques censés en réduire l'habitat. Les programmes actuels d'élimination de la THA (par l'OMS) et des TAA (sous la bannière de l'Union Africaine par le biais du PATTEC) tiendront compte de ces nouvelles données génétiques, qui contribuent au choix de la stratégie de lutte anti-vectorielle (contrôle ou élimination) et des méthodes utilisées (piégeage, SIT…). L'évolution spatiale de la THA depuis un siècle est également présentée avec un glissement du nord vers le sud des foyers, ayant comme conséquence une distribution de la THA en Afrique de l'Ouest limitée aux zones de mangrove côtières et de forêts dégradées, et son absence en savane. Il s'agira dans les prochaines années de comprendre la présence et l'absence des Trypanosomoses sur le terrain pour pouvoir prédire leur extension ou régression en tenant compte des interactions tsé-tsé/trypanosome et des prévisions démographiques et climatiques.

Summary:

Tsetse flies are the vectors of Human African Trypanosomosis (HAT otherwise known as sleeping sickness) and Animal African Trypanosomoses (AAT), which still constitute unacceptable problems for human health and economic loss in Subsaharan Africa. Studies conducted over the last 10 years on *G. palpalis* population genetics and on molecular identification of trypanosomes have shown that this species is organised in different sub-populations of small sizes. Each population is living in a limited space, and showing genetic differentiation from neighbouring populations. The degree of genetic subdivision may be related to the degree of spatial fragmentation occurring in its riverine or forested habitat. This tsetse species is able to survive in very densely populated areas such as some of the main urban centers of West Africa and is able to resist control operations surviving at very low densities. Taking into account recently published information on African population growth and climate change which should lead to tsetse habitat reduction in the coming years, G. palpalis now represents the most dangerous vector of the Trypanosomoses. Ongoing elimination programs (by WHO for HAT, by the African Union for AAT through the PATTEC program) recognize the importance of information on tsetse population genetics as a significant part of tsetse control strategies (elimination vs control), and control methods (baits, SIT…). The spatial evolution of sleeping sickness in the last century is also presented, showing a North to South shift of HAT foci, the disease being now restricted to mangrove and forest areas of West Africa. Next steps involve the understanding of the mechanisms explaining presence and absence of Trypanosomoses on the field, in order to anticipate their extension or extinction taking into account tsetse-trypanosome interactions in the context of demographic and climatic changes.