

SHORT COMMUNICATION

# Molecular characterization of the malaria vector *Anopheles gambiae s.s.* in Madagascar

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**Abstract.** *Anopheles gambiae s.s.* Giles (Diptera: Culicidae), the primary African malaria vector, has been characterized at the subspecies level in Madagascar, where only the molecular form S and haplotype gIA occur. The haplotype gIC proposed by other authors was not observed amongst the 35 mosquito genomes sequenced. These S/gIA characteristics are also found on the Comoros archipelago and in continental Africa.

**Key words.** *Anopheles gambiae*, ITS, IGS, malaria, molecular characterization, Madagascar.

*Anopheles gambiae s.s.* (Giles) is the most important malaria vector in sub-Saharan Africa and bears partial responsibility for millions of human deaths per year. Gentile *et al.* (2002) studied its genetic variability and defined three major haplotypes on the basis of sequences of the internal transcribed spacer (ITS) of the X-linked rDNA near the centromere region. Types I and II correspond, respectively, to the molecular forms S and M defined previously (Favia *et al.*, 1994). Type I is distributed throughout intertropical mainland Africa, type II is only observed in West Africa, and type III is restricted to the isolated island of São Tomé. In Madagascar, Gentile *et al.*, (2001) analysed five specimens of *An. gambiae s.s.* from one site, and interpreted them to be of type I and subtype IC, a minor variant of subtype IA with a single nucleotide difference. In order to establish a more ex-

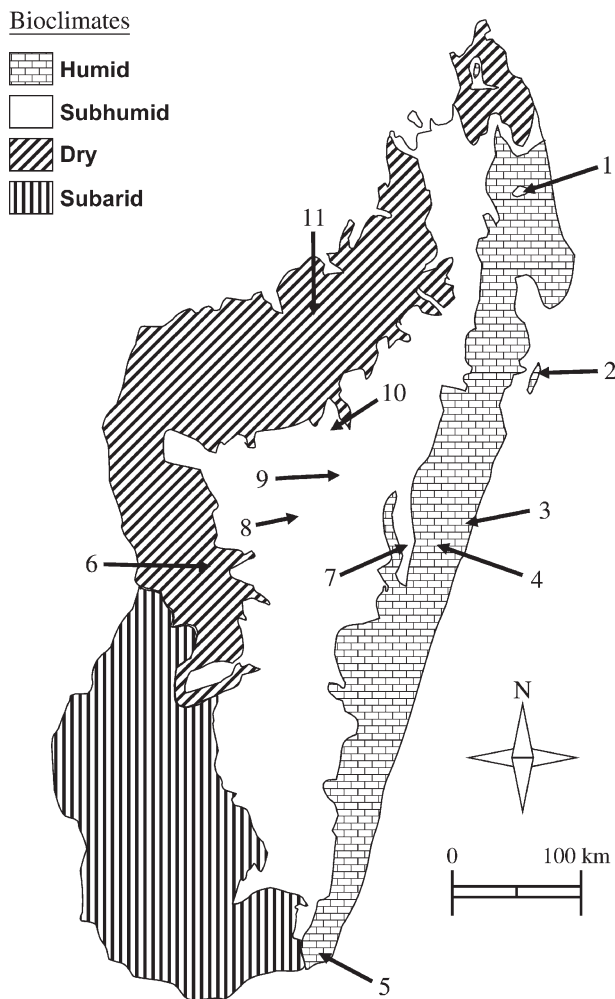
tensive distribution map of subtype IC and to investigate the existence of possible alternative subtype(s), we have extended the sampling and mapping of this mosquito in Madagascar. With comparable genetic methodologies, our findings agree only partially with the previously published observations.

Our sampling effort in Madagascar concerned 11 sites (Fig. 1 and Table 1) across the range of bioclimatic domains where *An. gambiae s.s.* is naturally distributed. The subarid domain is not known to harbour this species (Léong Pock Tsy *et al.*, 2003a). In order to eliminate other members of the *An. gambiae* species complex found in Madagascar (i.e. *An. arabiensis* Patton and *An. merus* Dönitz) from the samples, all mosquitoes caught were first identified to the species level by polymerase chain reaction (PCR) assays (Scott *et al.*, 1993). Determination of the

**Table 1.** Characteristics of *Anopheles gambiae* collected at particular locations.

Site	Name	Latitude	Longitude	Altitude (m)	Date of collection	No. IGS	No. ITS
1	Andapa Andogonzabe	14°65'	49°65'	480	October 2001	25	5
2	Sainte Marie Loukinty	16°88'	49°52'	10	January 2001	20	3
3	Brickaville Ambodivandrika	18°78'	49°00'	50	March 2000	18	3
4	Beforona	18°58'	48°15'	500	January 2003	15	7
5	Fort Dauphin Esana	25°04'	46°96'	50	July 2000	20	3
6	Miandrivazo Morarano	19°58'	45°47'	100	March 2001	20	3
7	Moramanga Saharevo	18°82'	48°10'	900	February 2000	12	3
8	Mahasolo Manarimofo	19°05'	46°23'	900	March 1999	1	1
9	Ankazobe	18°32'	47°12'	1300	May 1997 and March 1999	2	1
10	Mahatsinjo Manjakavaradrano	17°65'	46°97'	800	April 2001	6	3
11	Marovoay Tsararano	16°17'	46°67'	50	June 1999	19	3
Total						158	35

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**Fig. 1.** Location of the sampling sites of *Anopheles gambiae* s.s. mosquitoes in Madagascar, with an indication of the bioclimatic domains (after Cornet, 1974). The 11 site numbers correspond to those in Table 1.

molecular form was performed with a PCR assay targeting the intergenic spacer (IGS) of the X-linked rDNA (Favia *et al.*, 2001). The methodology used for ITS sequencing was that of Gentile *et al.* (2001). The molecular form of 158 *An. gambiae* s.s. female mosquitoes was identified, and 35 of these were selected for ITS sequencing (Table 1). The results were fully homogenous. All tested mosquitoes belonged to the molecular form S/type I. None of the tested mosquitoes presented the deletion of nucleotide G at position 847, which defines the subtype IC, and all were perfectly concordant with the sequence of haplotype gIA. These results agree with published observations from Madagascar concerning the determination of molecular form S/type I, but the total absence of gIC in our sample is puzzling. Based on the relative stability of the ITS sequences, it is hard to imagine how gIC might have been present in all five mosquitoes collected in Beforona (site 4, Fig. 1) in March 1992 (Gentile *et al.*, 2002) and yet none were found in our study. The most parsimonious hypothesis seems to question the validity of haplotype gIC.

Finally, *An. gambiae* s.s. of the molecular form S/type I and haplotype gIA has a widespread distribution across the whole of mainland tropical Africa, including East Africa (Kenya, Tanzania and Malawi) (Gentile *et al.*, 2001), the Comoros archipelago (Léong Pock Tsy *et al.*, 2003b), and Madagascar (this study). These observations are consistent with the view that *An. gambiae* s.s. dispersed out of Africa to the Indian Ocean islands, including the Comoros archipelago and Madagascar.

### Acknowledgements

We thank Jean-Claude Rakotoniaina for his technical assistance, Drs Gabriele Gentile and Gisella Caccone for their contributions to frank discussions, and Didier Fontenille, Pierre Kengne and Arthur Talman for improving the final version of the manuscript. This work was supported by the Ministère Français de la Jeunesse, de l'Éducation et de la Recherche through the Programme Pal + and its African *Anopheles* Network, the Strategic *Anopheles* Horizontal Programme of the Institut Pasteur, and the Institut de Recherche pour le Développement.

### References

- Cornet, A. (1974) *Essai de Cartographie Bioclimatique À Madagascar, Carte À 1/2 000 000 et Notice*. Editions ORSTOM, Paris. <http://www.mobot.org/MOBOT/research/madagascar/maps/bc5tif.jpg>. [Accessed 03 June 2006]
- Favia, G., Dimopoulos, G., della Torre, A., Touré, Y.T., Coluzzi, M. & Louis, C. (1994) Polymorphisms detected by random PCR distinguish between different chromosomal forms of *Anopheles gambiae*. *Proceedings of the National Academy of Sciences USA*, **91**, 10315–10319.
- Favia, G., Lanfrancotti, A., Spanos, L., Sindén-Kiamos, I. & Louis, C. (2001) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. *Insect Molecular Biology*, **10**, 19–23.
- Gentile, G., della Torre, A., Maegga, B., Powell, J.R. & Caccone, A. (2002) Genetic differentiation in the African malaria vector, *Anopheles gambiae* s.s. and the problem of taxonomic status. *Genetics*, **161**, 1561–1578.
- Gentile, G., Slotman, M., Ketmaier, V., Powell, J.R. & Caccone, A. (2001) Attempts to molecularly distinguish cryptic taxa in *Anopheles gambiae* s.s. *Insect Molecular Biology*, **10**, 25–32.
- Léong Pock Tsy, J.M., Duchemin, J.B., Marrama, L., Rabarison, P., Le Goff, G., Rajaonarivelo, V. & Robert, V. (2003a) Distribution of the species of the *Anopheles gambiae* complex, and first evidences of *An. merus* as malaria vector in Madagascar. *Malaria Journal*, **2**, 33.
- Léong Pock Tsy, J.M., Elissa, N., Ouledi, A., Ariey, F., Duchemin, J.B. & Robert, V. (2003b) Caractérisation moléculaire des moustiques du complexe *Anopheles gambiae* à Mayotte et à Grande Comore. *Parasite*, **10**, 273–276. [Erratum *Parasite*, **10**, 370.]
- Scott, J.A., Brogdon, W.G. & Collins, F.H. (1993) Identification of single specimens of *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, **49**, 520–529.

Accepted 22 March 2006